Electronic Supplementary Information

Attenuating HIV Tat/TAR-mediated protein expression by exploring the side chain length of positively charged residues

Cheng-Hsun Wu, Yi-Ping Chen, Shing-Lung Liu, Fan-Ching Chien, Chung-Yuan Mou, and Richard P. Cheng*

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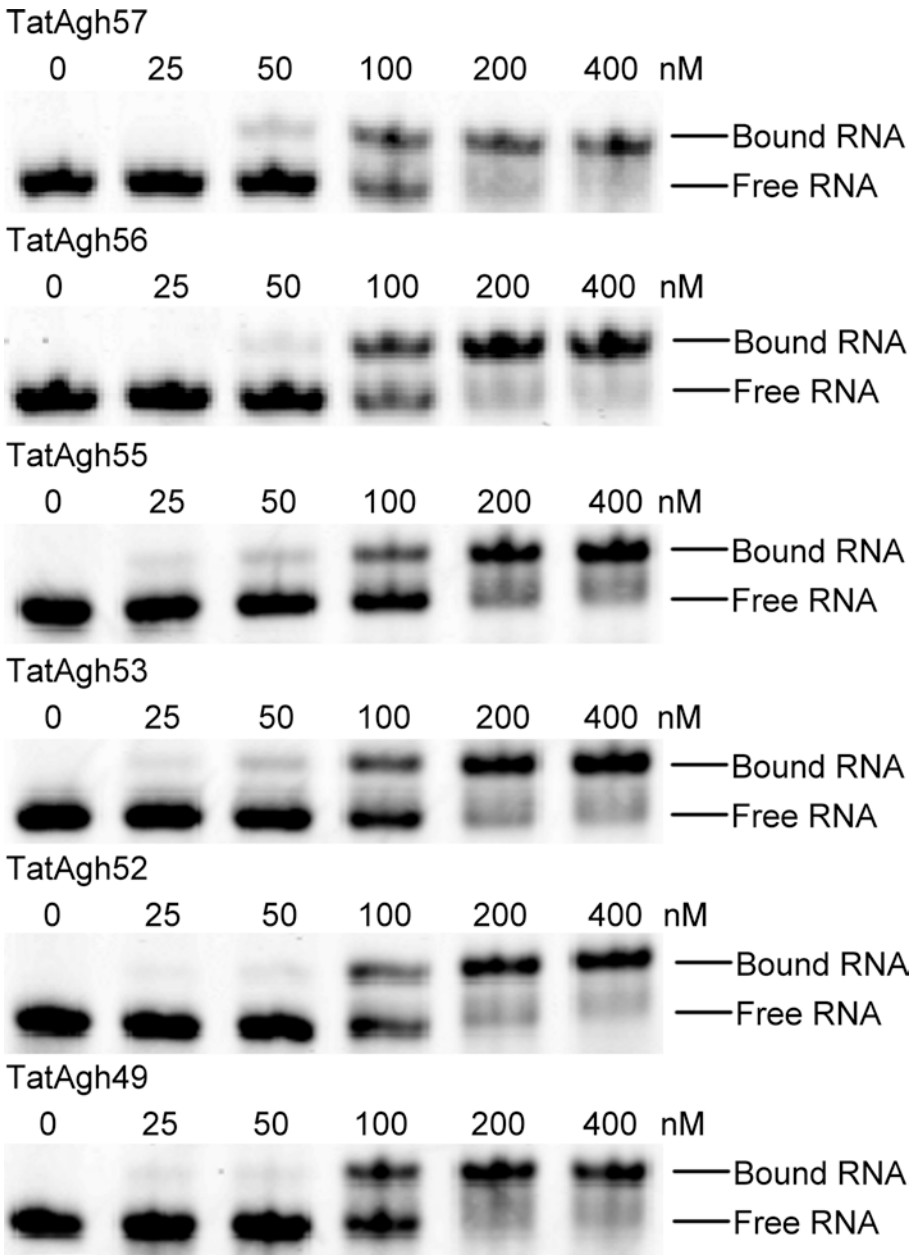

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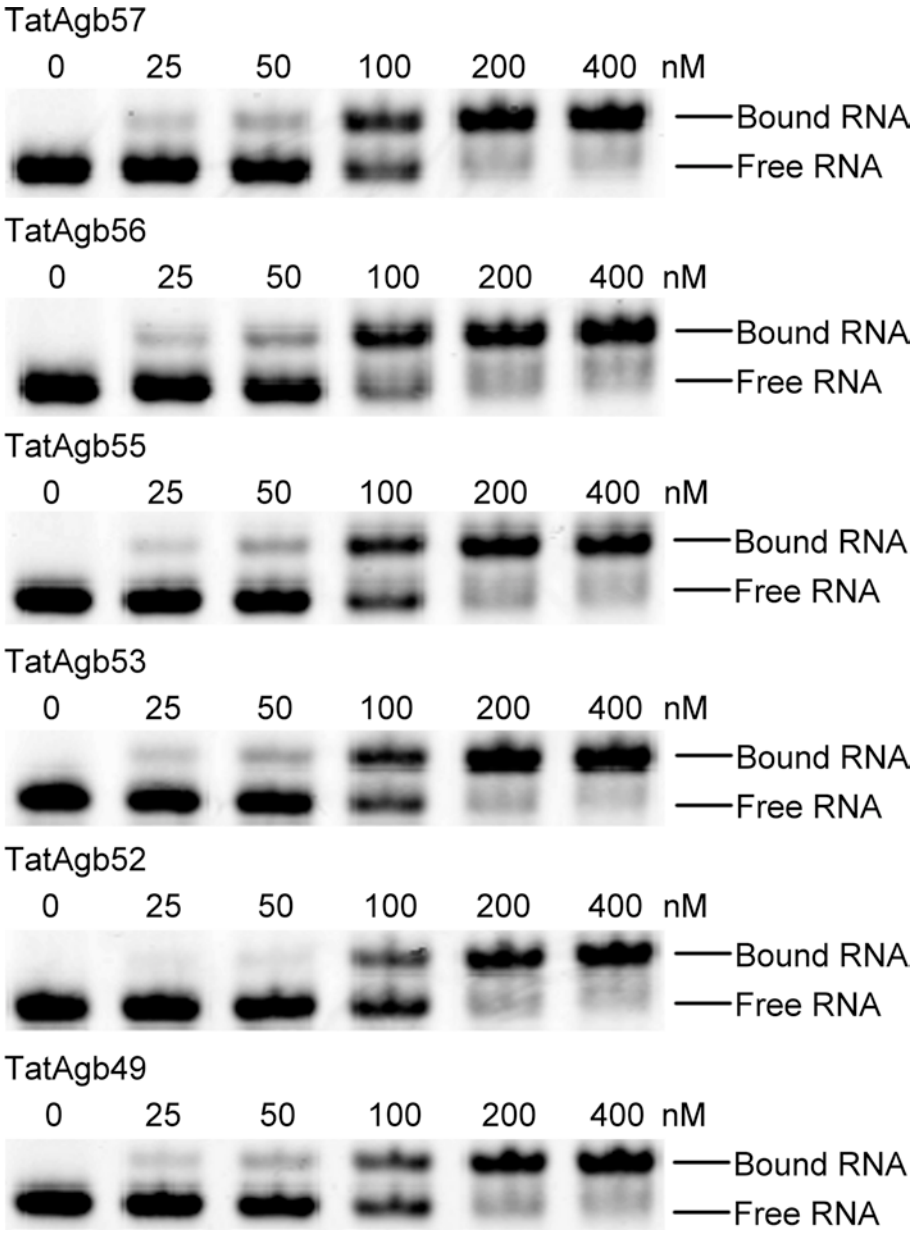

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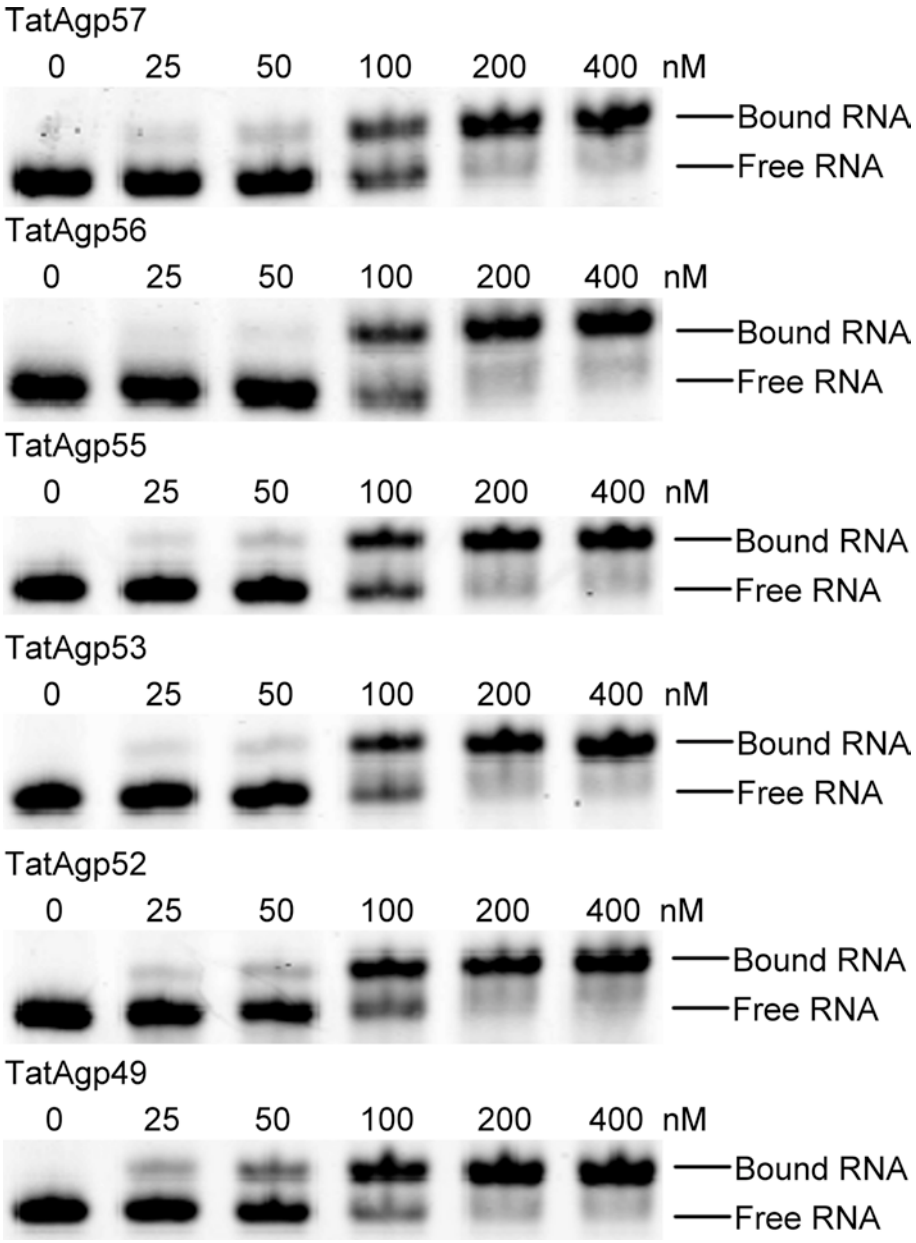

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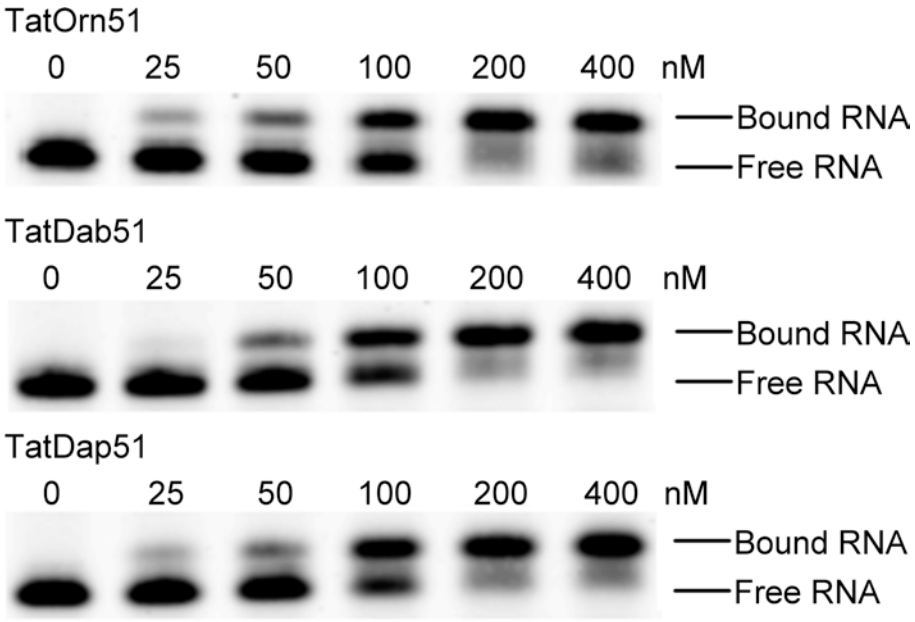

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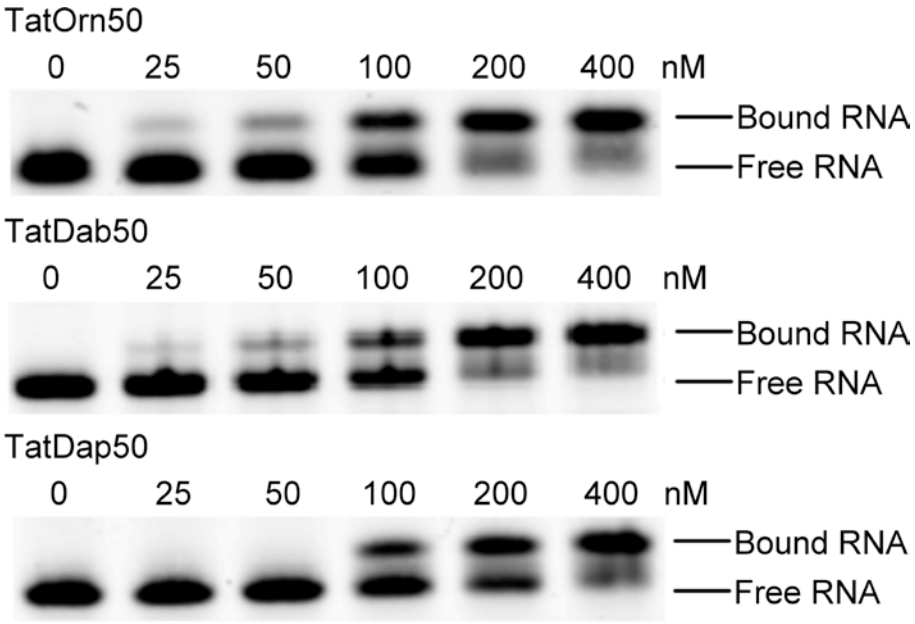

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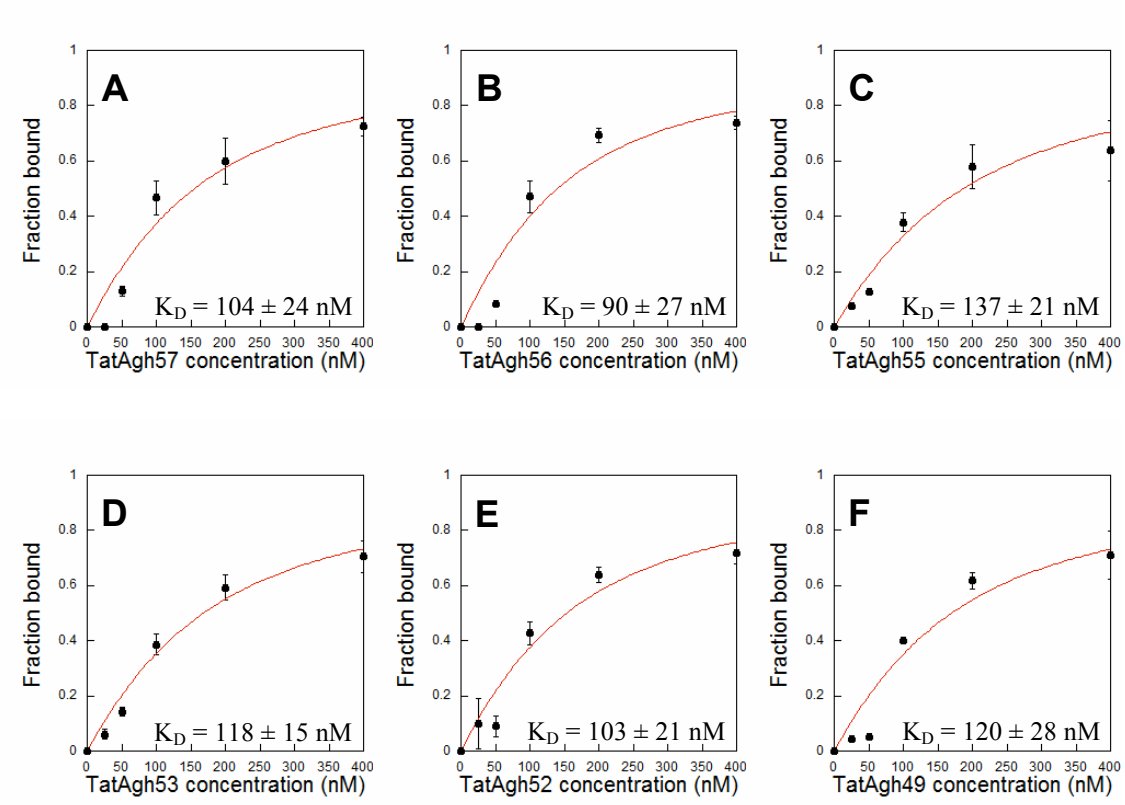


Figure S6. Global fitting of the electrophoretic mobility shift assay results for peptides TatAgh*N* with 100 nM fluorescein-labeled HIV TAR RNA in the presence of 10 μg/mL poly-dIdC. The black dots with standard deviations represent data from at least three experiments, and the red curves represent the fits based on 1:1 binding stoichiometry for deriving the apparent dissociation constants.

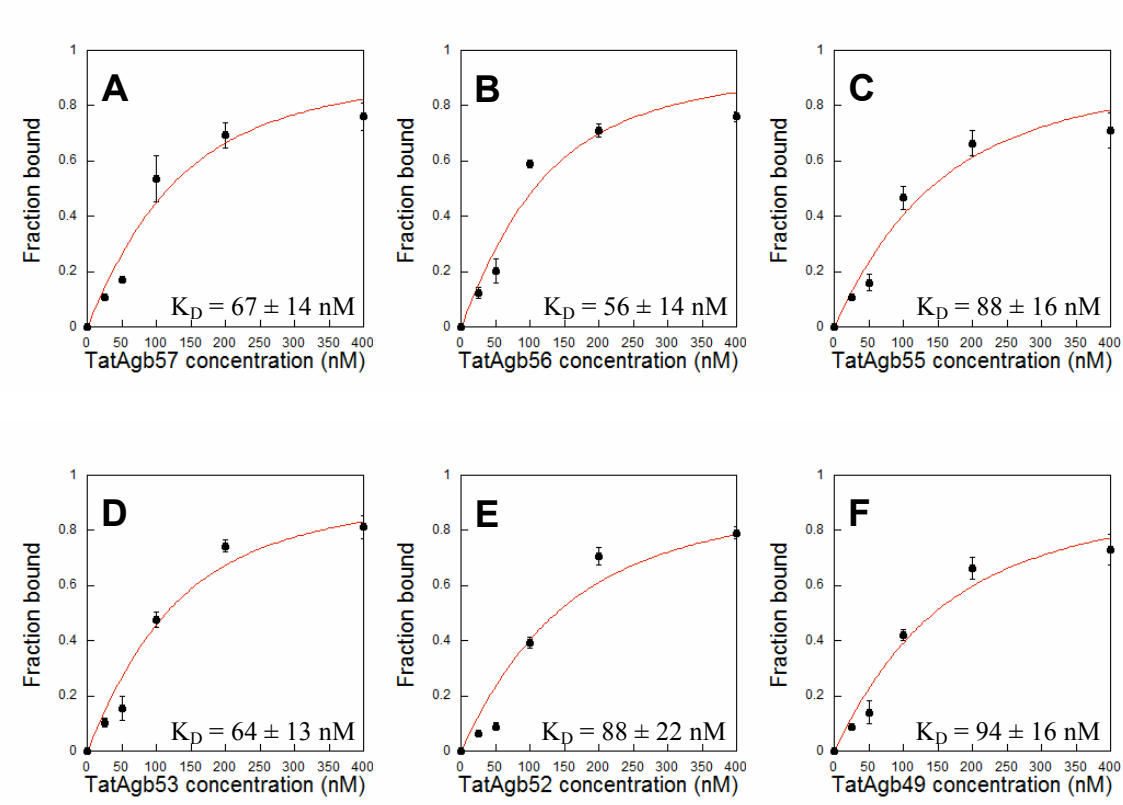
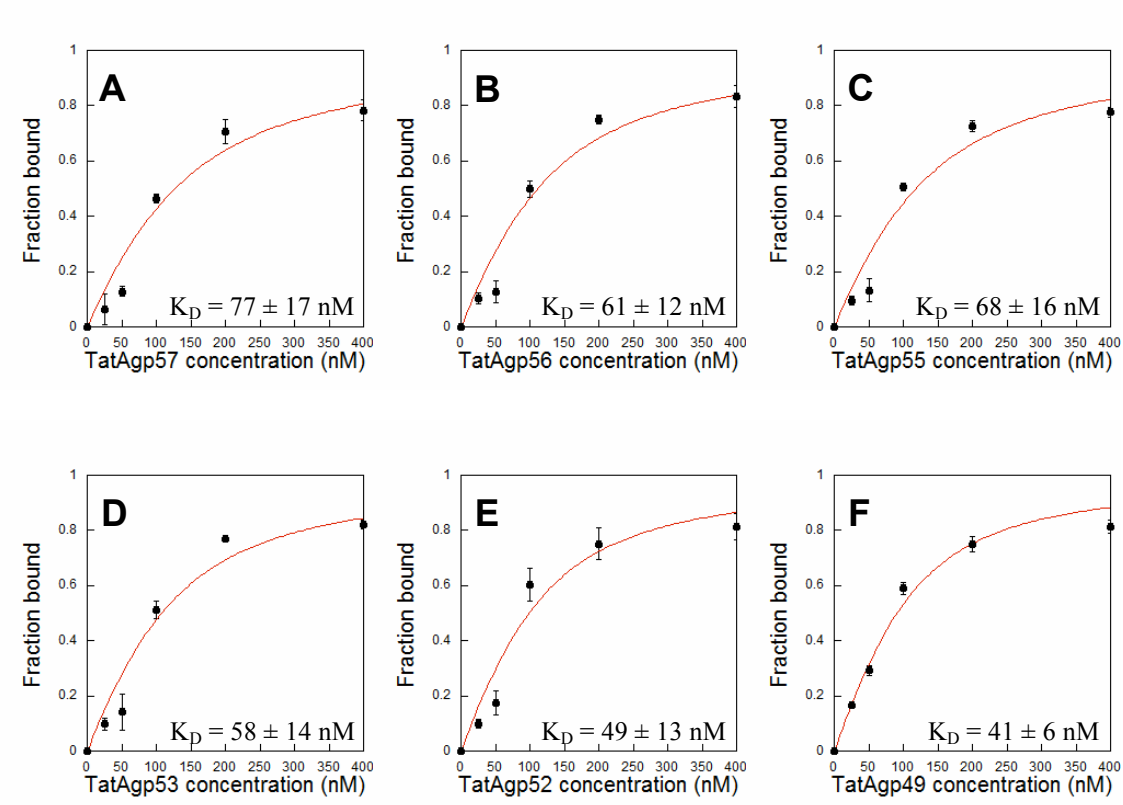


Figure S7. Global fitting of the electrophoretic mobility shift assay results for peptides TatAgb*N* with 100 nM fluorescein-labeled HIV TAR RNA in the presence of 10 μg/mL poly-dIdC. The black dots with standard deviations represent data from at least three experiments, and the red curves represent the fits based on 1:1 binding stoichiometry for deriving the apparent dissociation constants.



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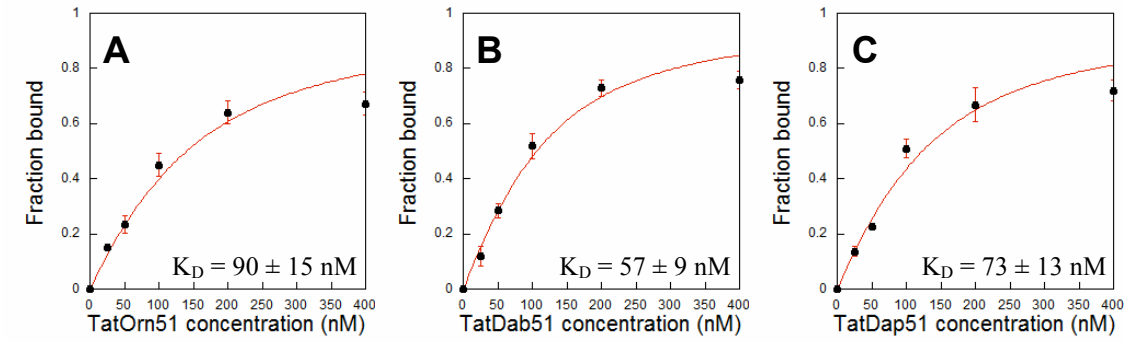


Figure S9. Global fitting of the electrophoretic mobility shift assay results for peptides TatZbb*51* (Zbb = Orn, Dab, and Dap) with 100 nM fluorescein-labeled HIV TAR RNA in the presence of 10 μg/mL poly-dIdC. The black dots with standard deviations represent data from at least three experiments, and the red curves represent the fits based on 1:1 binding stoichiometry for deriving the apparent dissociation constants.

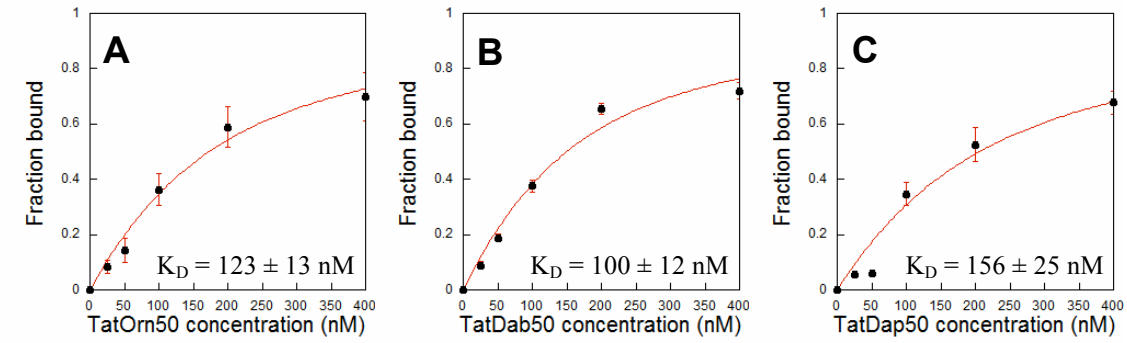


Figure S10. Global fitting of the electrophoretic mobility shift assay results for peptides TatZbb*50* (Zbb = Orn, Dab, and Dap) with 100 nM fluorescein-labeled HIV TAR RNA in the presence of 10 μg/mL poly-dIdC. The black dots with standard deviations represent data from at least three experiments, and the red curves represent the fits based on 1:1 binding stoichiometry for deriving the apparent dissociation constants.

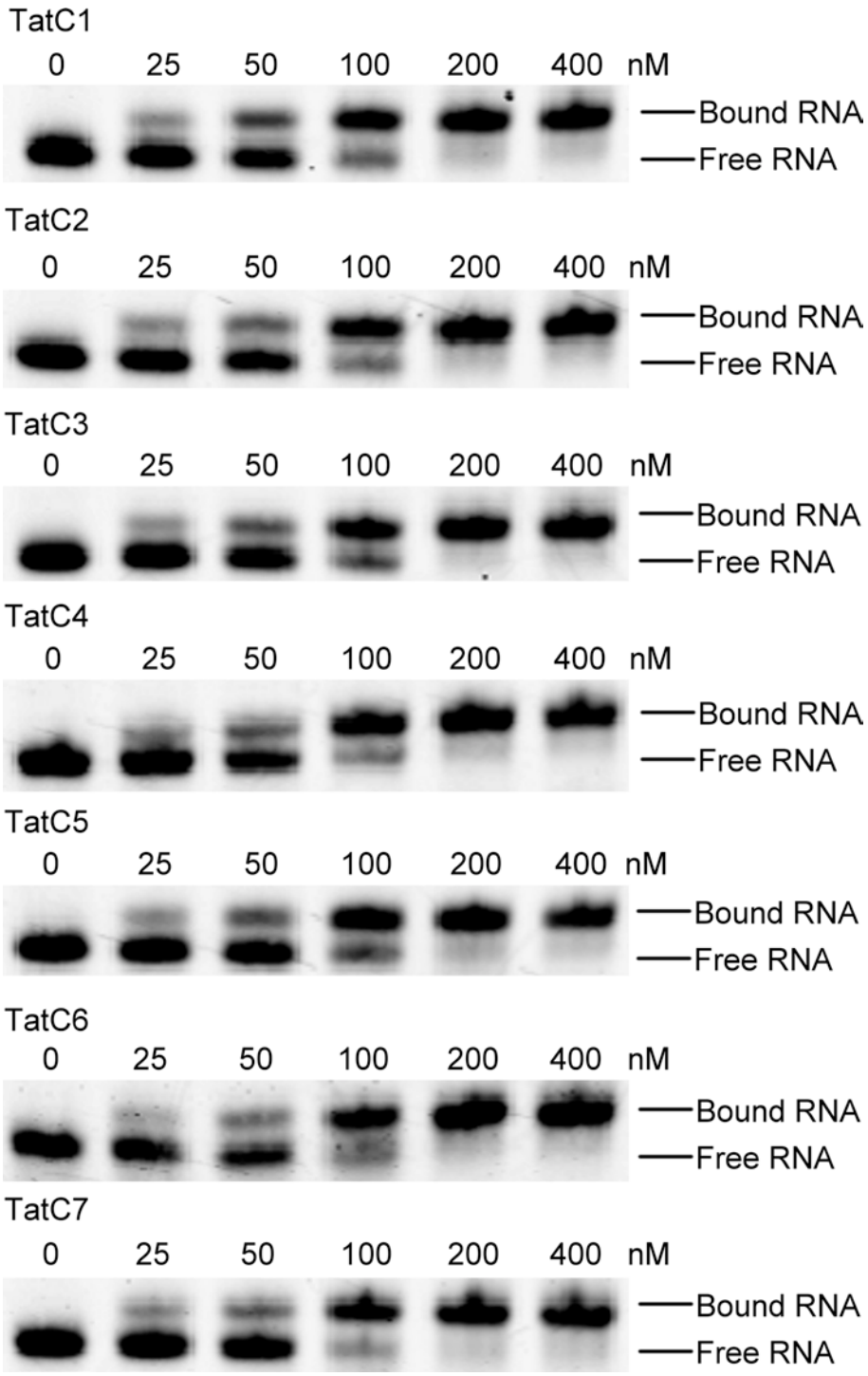


Figure S11. Gel images of electrophoretic mobility shift assays for TatCX peptides with 100 nM fluorescein-labeled HIV TAR RNA in the presence of 10 μg/mL poly-dIdC.

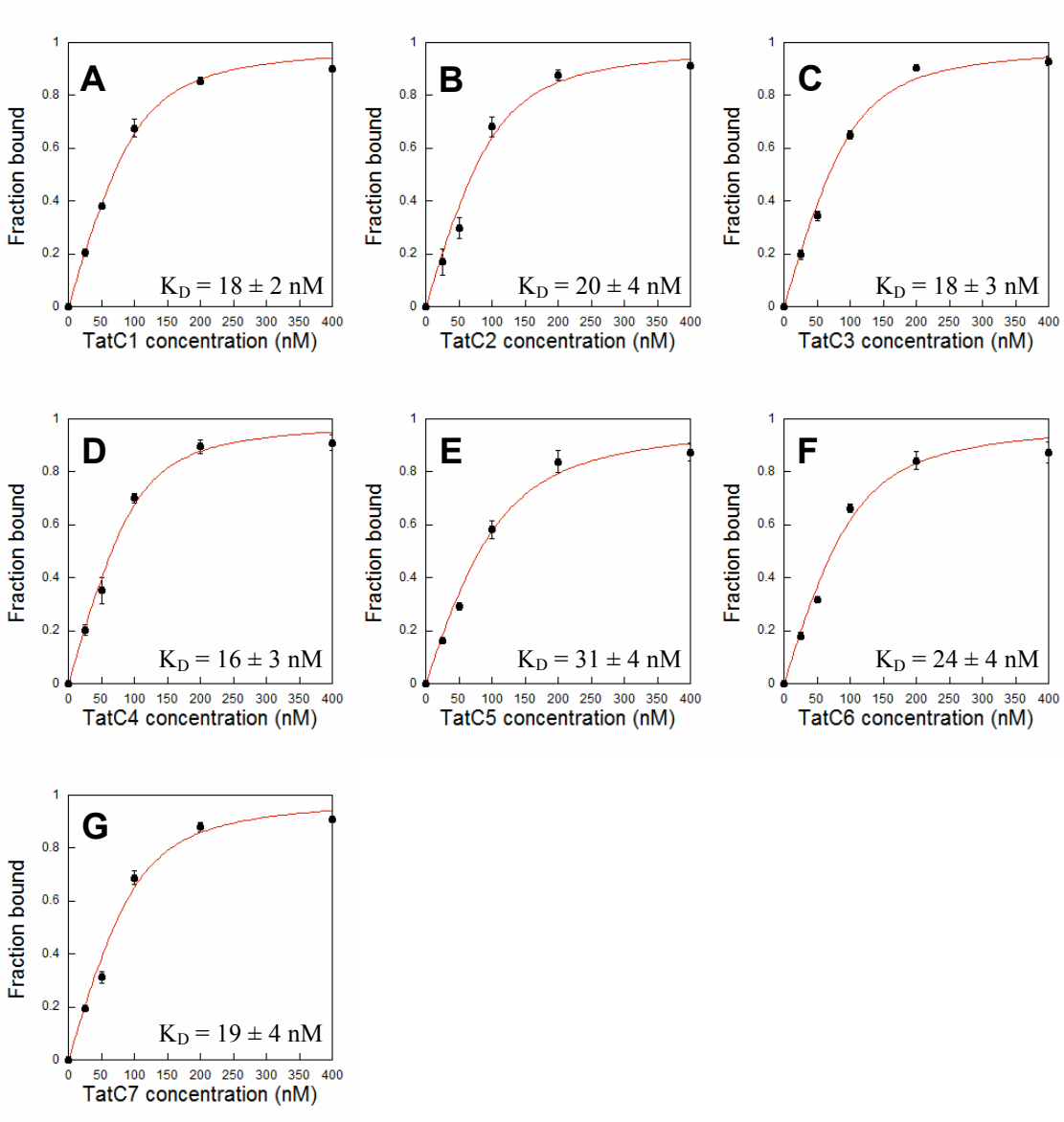


Figure S12. Global fitting of the electrophoretic mobility shift assay results for peptides TatCX with 100 nM fluorescein-labeled HIV TAR RNA in the presence of 10 μg/mL poly-dIdC. The black dots with standard deviations represent data from at least three experiments, and the red curves represent the fits based on 1:1 binding stoichiometry for deriving the apparent dissociation constants.

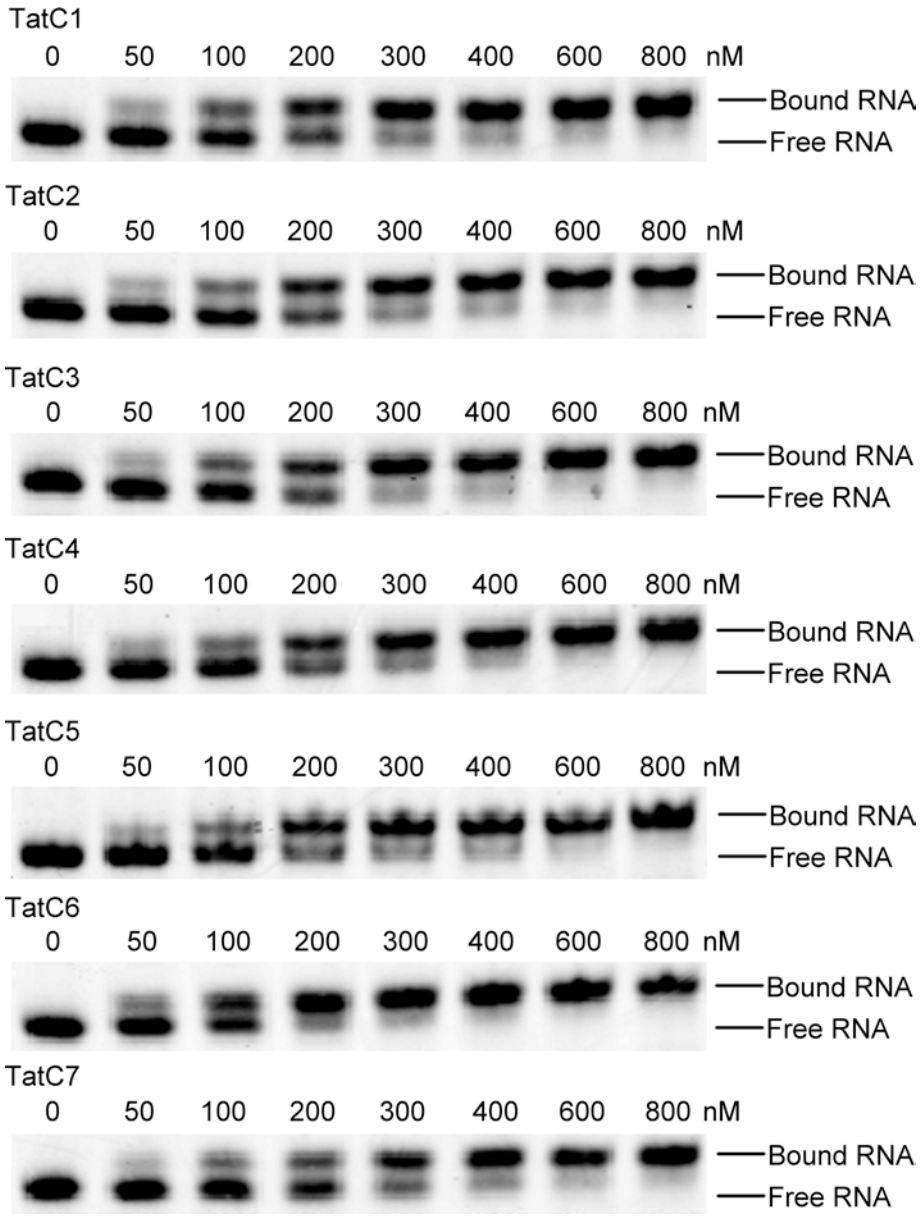


Figure S13. Gel images of electrophoretic mobility shift assays for TatCX peptides with 100 nM fluorescein-labeled HIV TAR RNA in the presence of 10 μg/mL bulk *E. coli* tRNA.

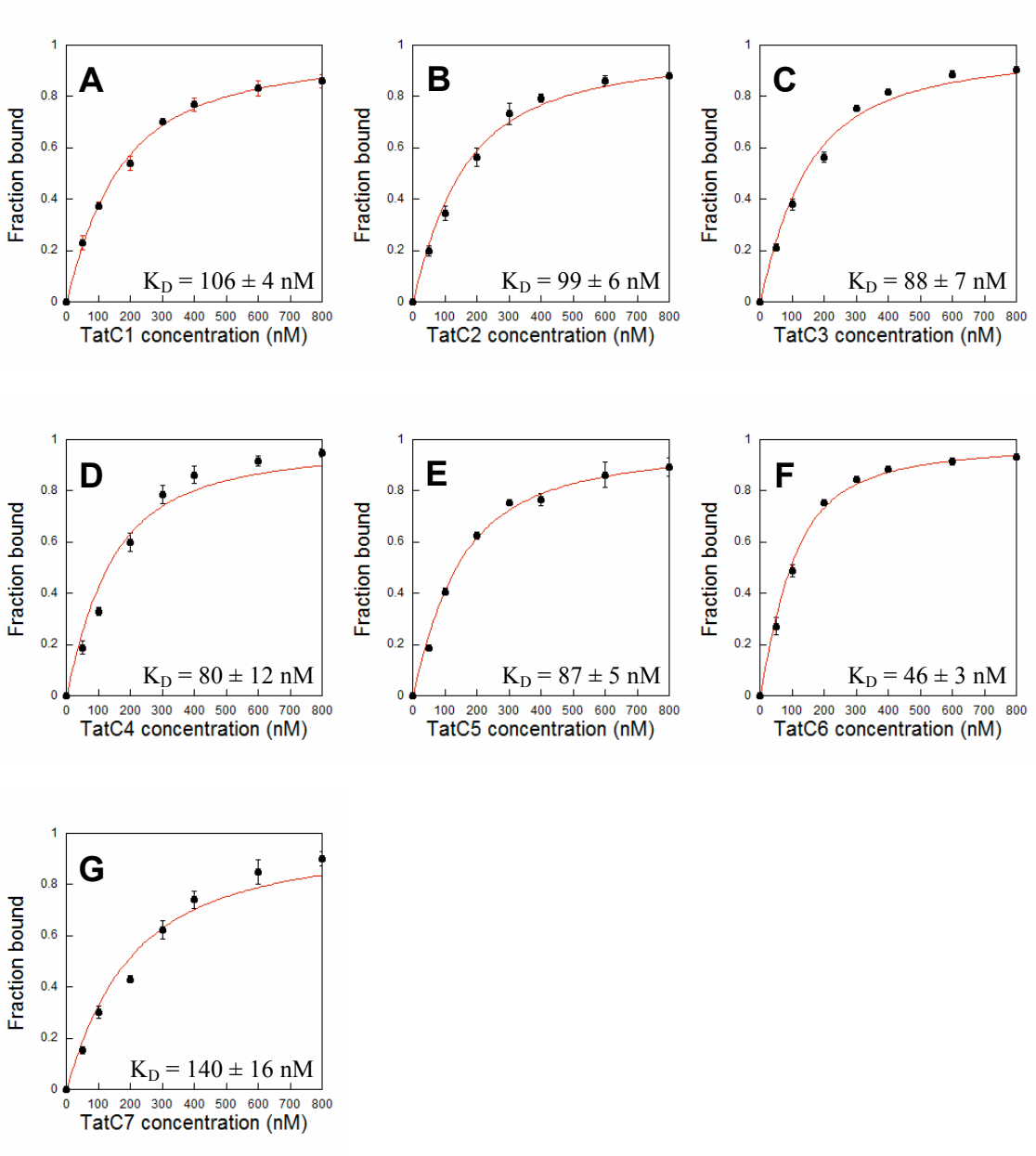


Figure S14. Global fitting of the electrophoretic mobility shift assay results for peptides TatCX with 100 nM fluorescein-labeled HIV TAR RNA in the presence of 10 μg/mL bulk *E. coli* tRNA. The black dots with standard deviations represent data from at least three experiments, and the red curves represent the fits based on 1:1 binding stoichiometry for deriving the apparent dissociation constants.

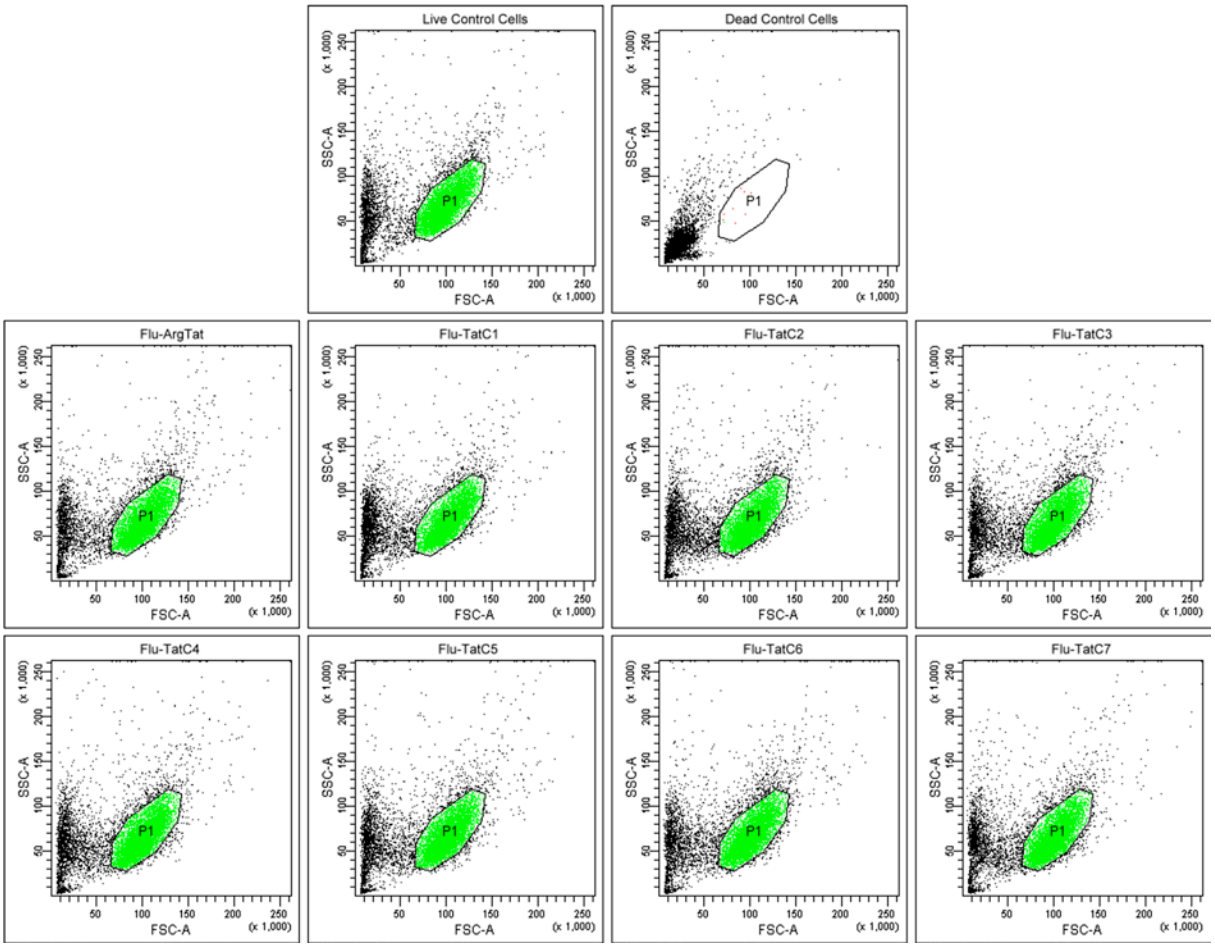


Figure S15. Flow cytometry results showing the side scattered light plotted against the forward scattered light for live control cells, dead control cells, and cells incubated with 7 μM Flu-ArgTat and Flu-TatCX peptides. The gate used to restrict the population of cells analyzed is shown and labeled as P1.

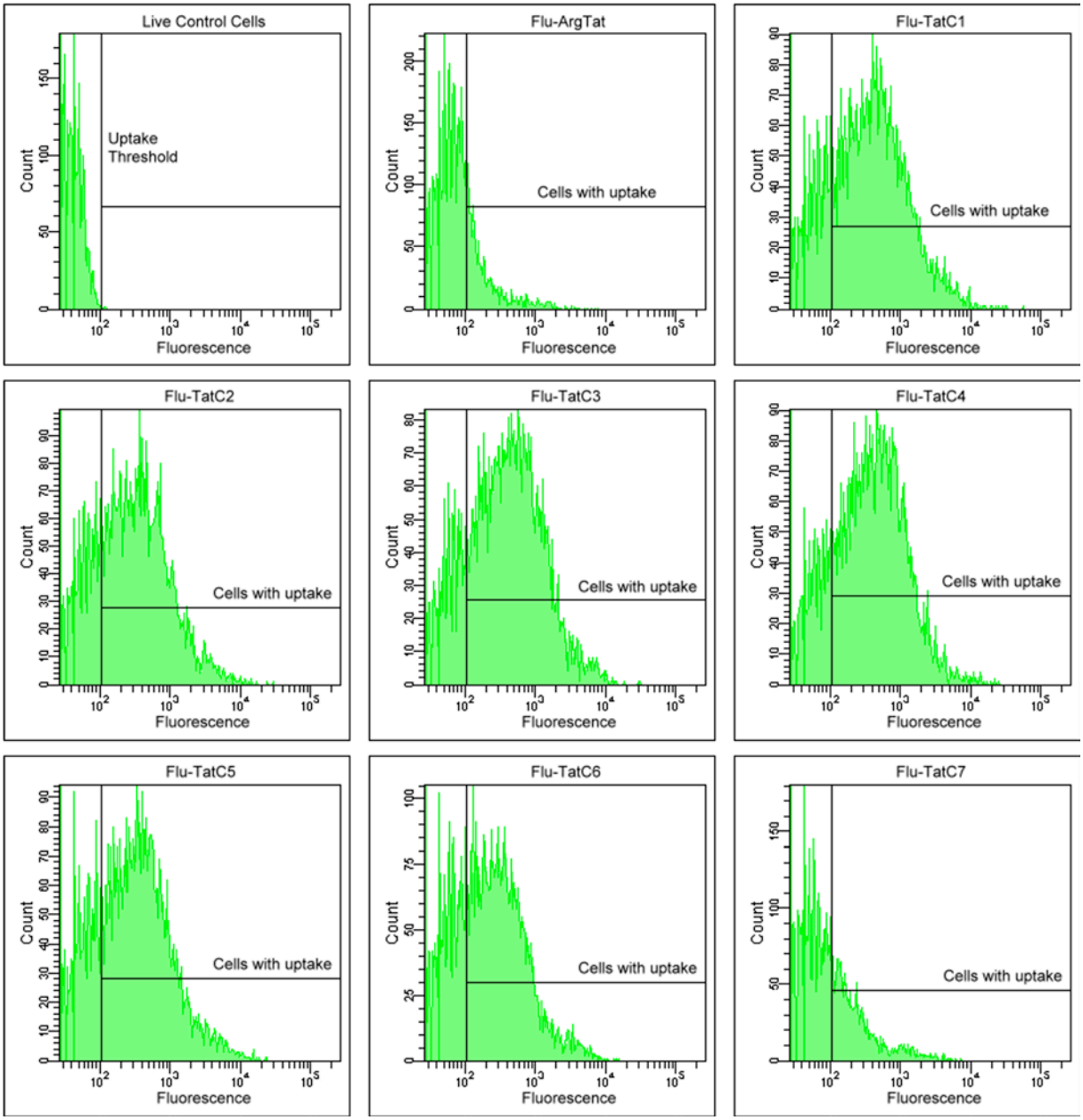


Figure S16. Flow cytometry results showing the fluorescence signal for live control cells, and cells incubated with 7 μM Flu-ArgTat and Flu-TatCX peptides for 15 minutes at 37 ^{o}C. The threshold for determining cellular uptake is also depicted.

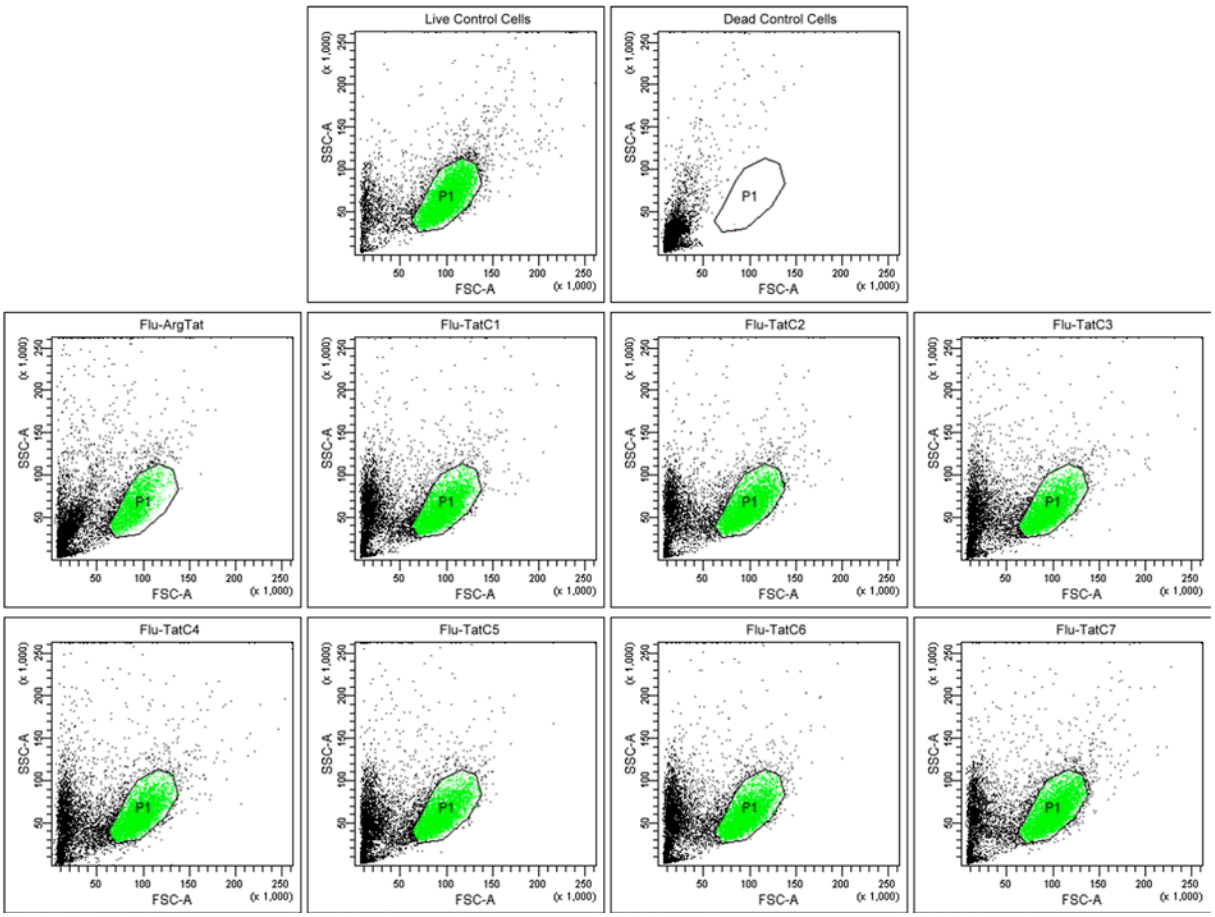


Figure S17. Flow cytometry results showing the side scattered light plotted against the forward scattered light for live control cells, dead control cells, and cells incubated with 120 μM Flu-ArgTat and Flu-TatCX peptides. The gate used to restrict the population of cells analyzed is shown and labeled as P1.

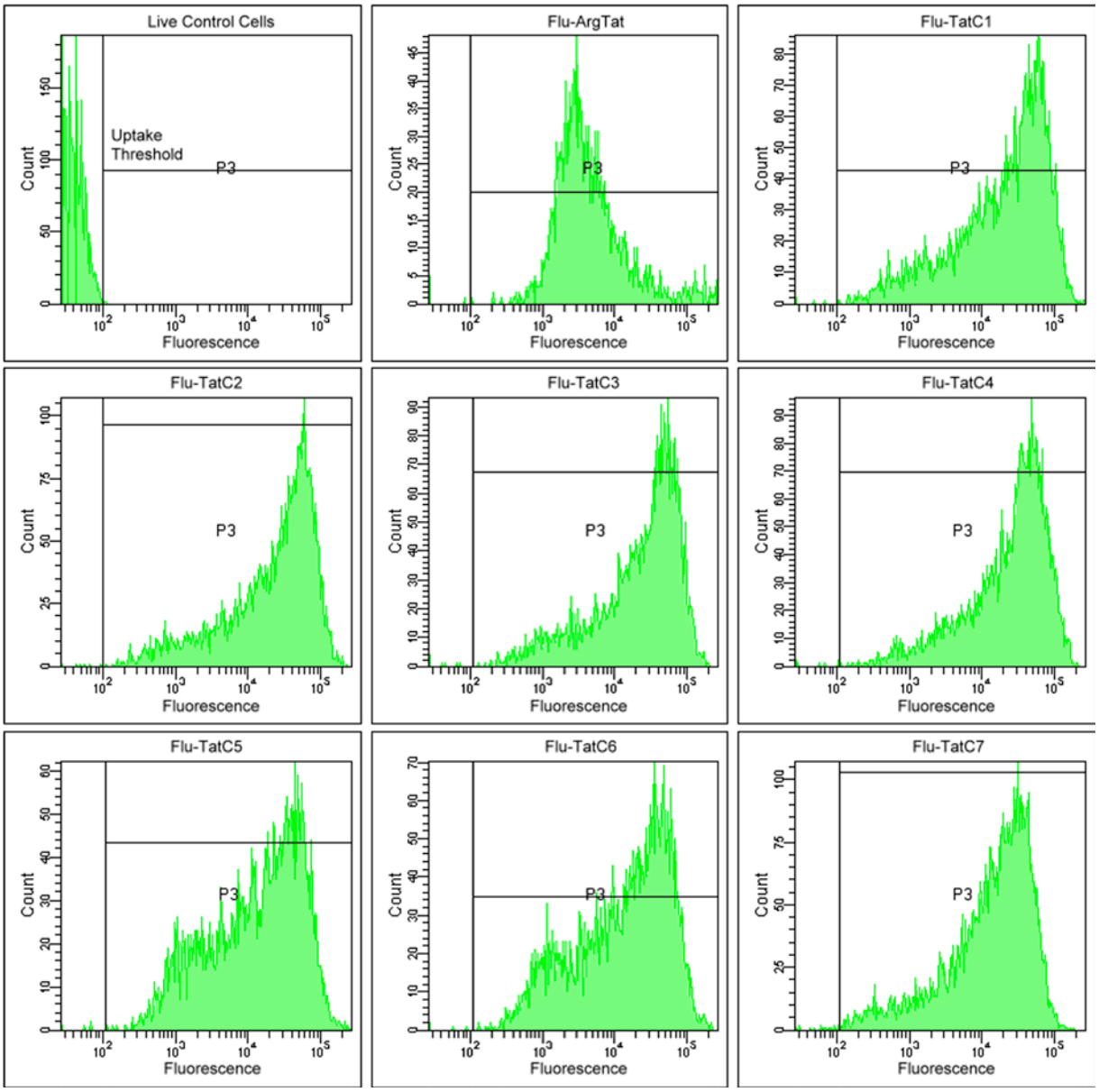


Figure S18. Flow cytometry results showing the fluorescence signal for live control cells, and cells incubated with 120 μM Flu-ArgTat and Flu-TatCX peptides for 15 minutes at 37 ^{o}C. The threshold for determining cellular uptake is also depicted.

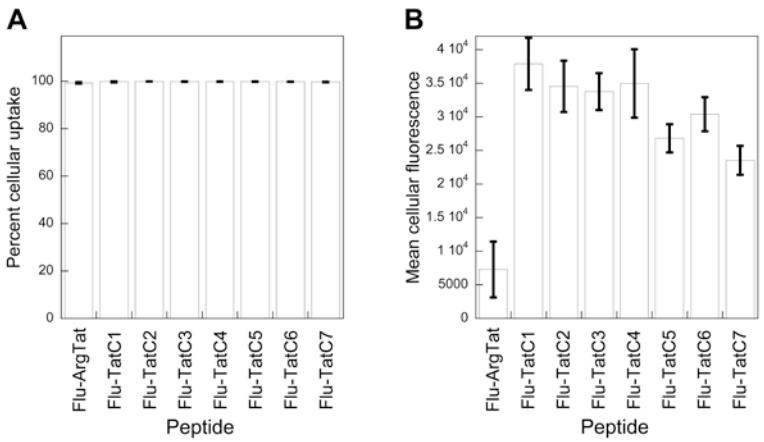


Figure S19. Flow cytometry results for cellular uptake of Flu-TatCX peptides into Jurkat cells. Percent cellular uptake (A) and mean cellular fluorescence (B) upon incubating with 120 μM of Flu-TatCX peptides at 37 ^{o}C for 15 minutes.

Peptide	Sequence
Flu-ArgTat	Flu-βAla-Tyr-Gly-Arg_{49}-Lys_{50}-Lys_{51}-Arg_{52}-Arg_{53}-Gln-Arg_{55}-Arg_{56}-Arg_{57}-NH_2
Flu-TatOrn51	Flu-βAla-Tyr-Gly-Arg_{49}-Lys_{50}-Orn_{51}-Arg_{52}-Arg_{53}-Gln-Arg_{55}-Arg_{56}-Arg_{57}-NH_2
Flu-TatDab51	Flu-βAla-Tyr-Gly-Arg_{49}-Lys_{50}-Dab_{51}-Arg_{52}-Arg_{53}-Gln-Arg_{55}-Arg_{56}-Arg_{57}-NH_2
Flu-TatDap51	Flu-βAla-Tyr-Gly-Arg_{49}-Lys_{50}-Dap_{51}-Arg_{52}-Arg_{53}-Gln-Arg_{55}-Arg_{56}-Arg_{57}-NH_2

Flu = 6-carboxy-fluorescein; βAla = β-alanine

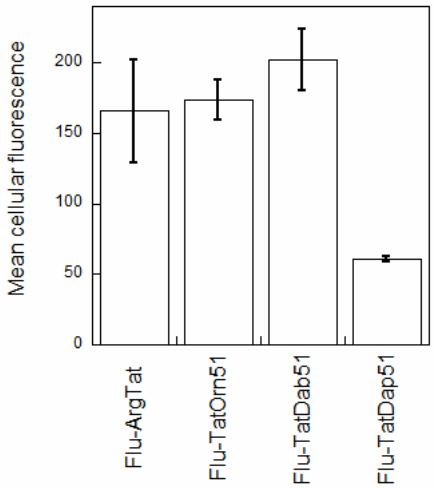
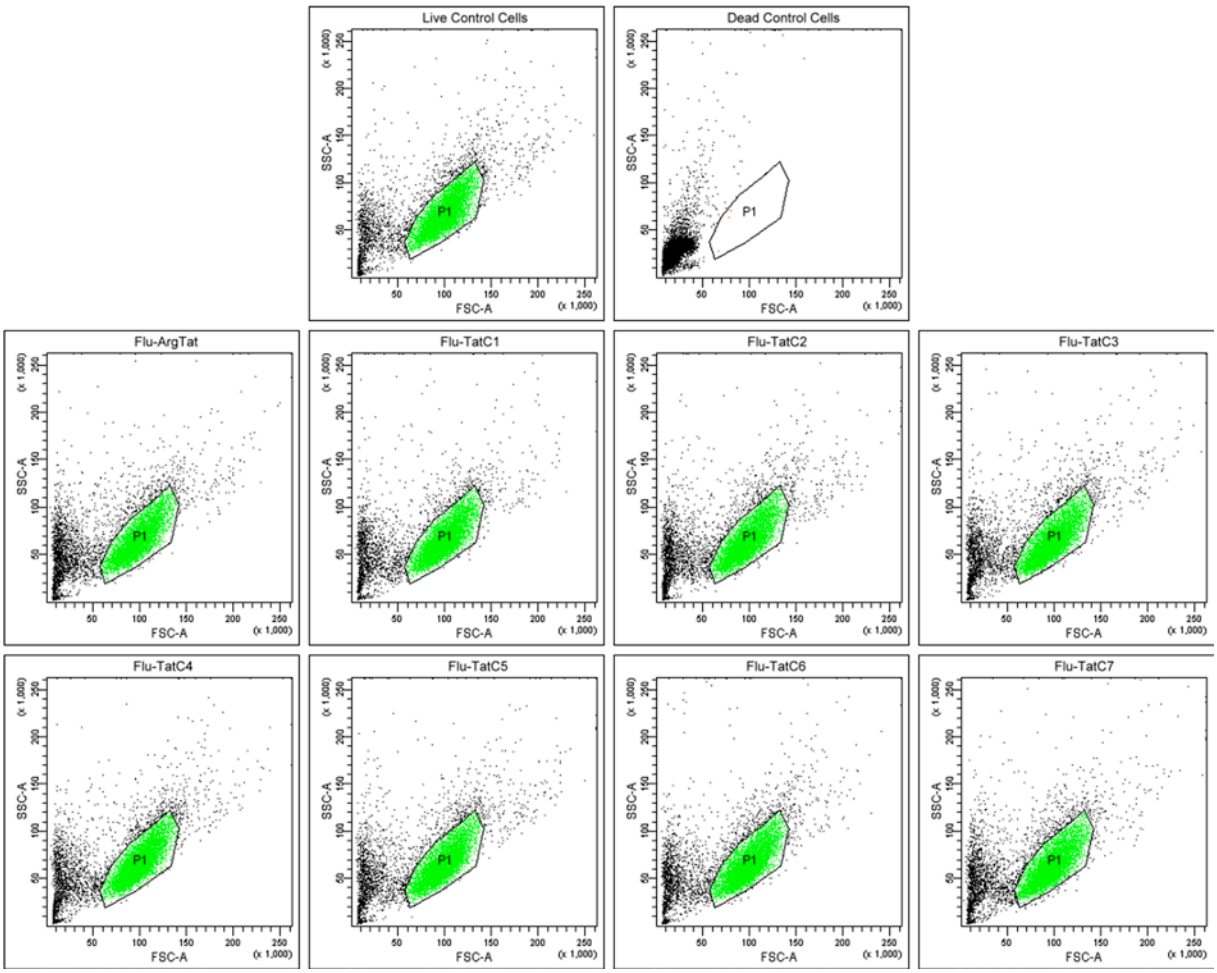


Figure S20. Flow cytometry results for cellular uptake of Flu-TatZbb51 peptides (Zbb = Lys, Orn, Dab, and Dap) into Jurkat cells in the presence of fetal bovine serum at 37 ˚C. Mean cellular fluorescence upon incubation with 7 μM peptide.



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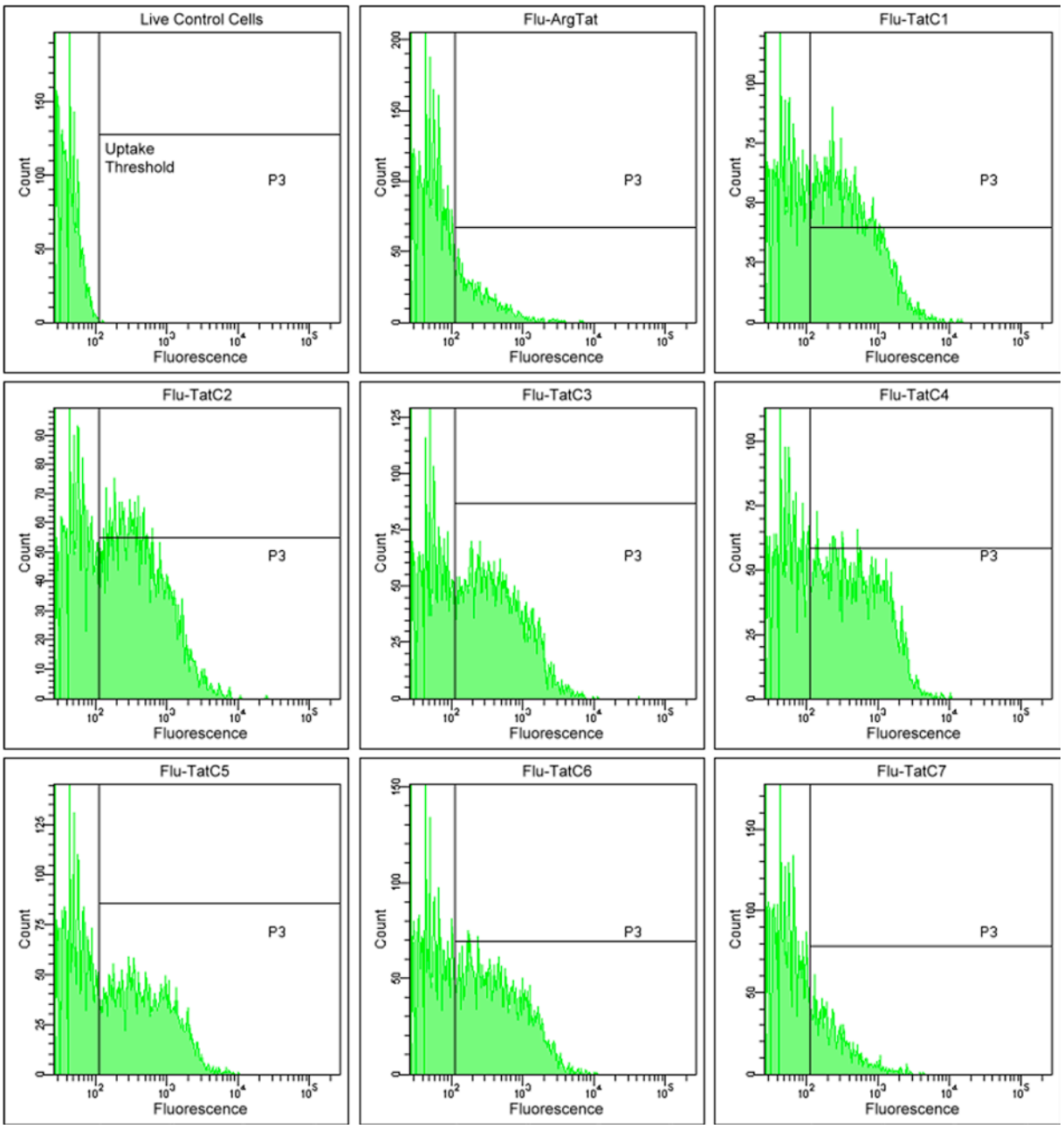

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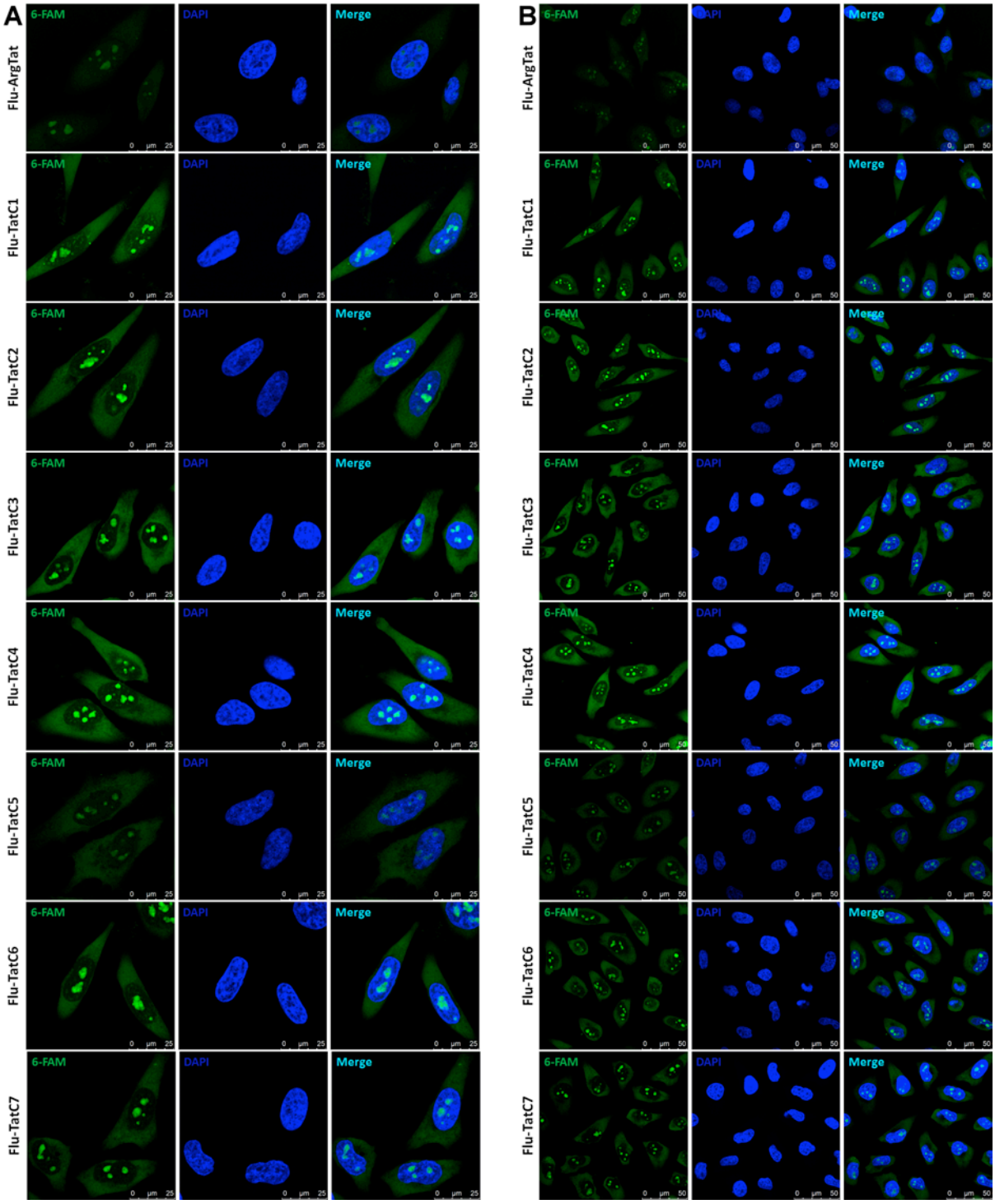


Figure S23. Confocal microscopy images of HeLa cells incubated with 10 μM Flu-ArgTat and Flu-TatCX for 4 hours (left column). The cells were fixed and stained with the DNA-specific DAPI (center column) to obtain the merged nuclear co-localization images (right column). Zoomed-in image (A), and zoomed-out image (B).

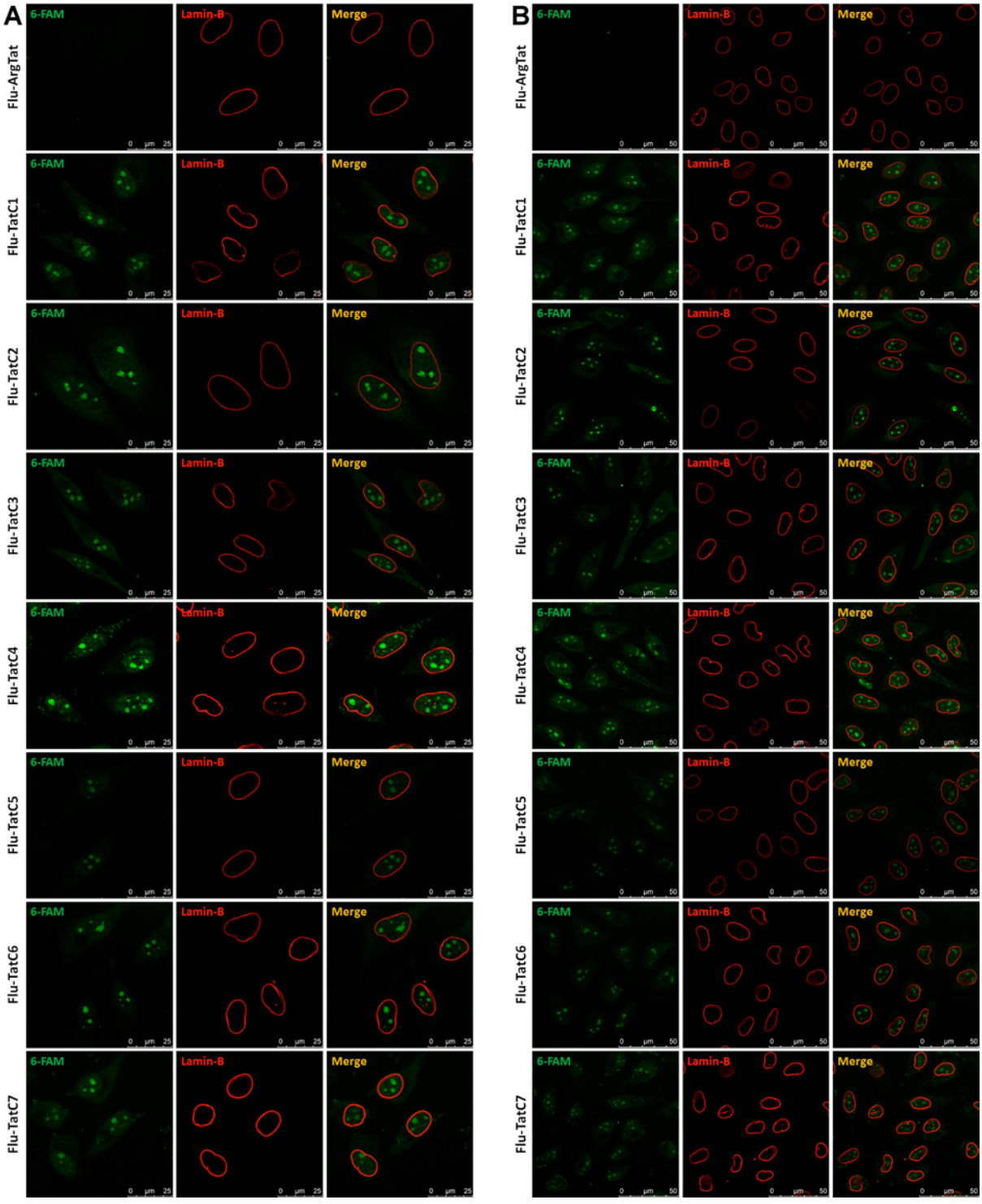


Figure S24. Confocal microscopy images of HeLa cells incubated with 10 μM Flu-ArgTat and Flu-TatCX for 24 hours (left column). The cells were fixed, labeled with antibody against Lamin B receptor and then Alexa Fluor 647-conjugated secondary anti-goat antibody to define the boundary of inner nuclear membrane (center column) and observed by confocal microscopy. Zoomed-in image (A), and zoomed-out image (B).

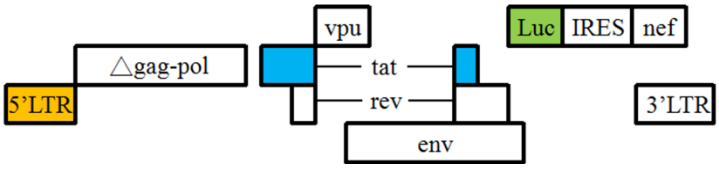


Figure S25. Genomic organization of the lentiviral vector was intergrated into the host genome.

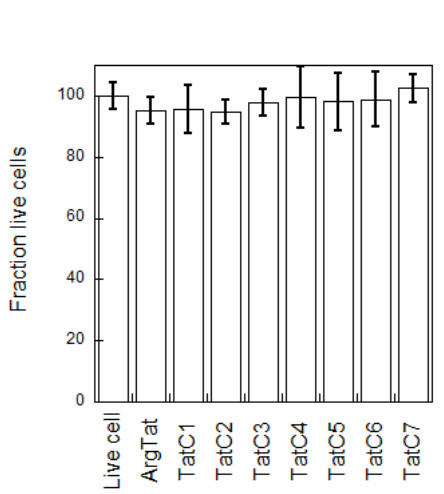


Figure S26. The cell viability of ArgTat and TatCX at 120 μM for 24 hours at 37 ^{o}C were determined by WST-1 assays.

Table S1. Apparent dissociation constants for the binding of individually substituted Tat-derived peptides containing Arg analogs with varying side chain lengths to HIV TAR RNA in the presence of poly(dI-dC).

	Residue		
	Agh	Agb	Agp
Position	K_D (nM)	K_D (nM)	K_D (nM)
57	104 ± 24	67 ± 14	77 ± 17
56	90 ± 27	56 ± 14	61 ± 15
55	137 ± 21	88 ± 16	68 ± 16
53	118 ± 15	64 ± 13	58 ± 14
52	103 ± 21	88 ± 22	49 ± 13
49	120 ± 28	94 ± 16	41 ± 6

The apparent dissociation constants were derived from the experimental data assuming a 1:1 binding stoichiometry. ArgTat K_D = 67 ± 19 nM.

Table S2. Apparent dissociation constants for the binding of individually substituted Tat-derived peptides containing Lys analogs with varying side chain lengths to HIV TAR RNA in the presence of poly(dI-dC).

	Residue		
Position	Orn	Dab	Dap
51	90 ± 15	57 ± 9	73 ± 13
50	123 ± 13	100 ± 12	156 ± 25

The apparent dissociation constants were derived from the experimental data assuming a 1:1 binding stoichiometry. ArgTat K_D = 67 ± 19 nM.

Table S3. Apparent dissociation constants for the binding of the TatCX peptides to HIV TAR RNA in the presence of poly(dI-dC) or bulk *E.coli* tRNA.

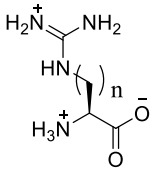
	Apparent K_D (nM)	
peptide	poly(dI-dC)[a]	tRNA[b]
ArgTat	67 ± 19	260 ± 30
TatC1	18 ± 2	106 ± 4
TatC2	20 ± 4	99 ± 6
TatC3	18 ± 3	88 ± 7
TatC4	16 ± 3	80 ± 12
TatC5	31 ± 4	87 ± 5
TatC6	24 ± 4	46 ± 3
TatC7	19 ± 4	140 ± 16

[a] The apparent K_D was determined in the presence of 10 μg/mL poly(dI-dC). [b] The apparent K_D was determined in the presence of 10 μg/mL bulk *E. coli* tRNA.

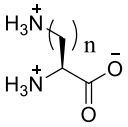
Table S4. Sequence of fluorescein-labeled potentially optimal Tat-derived peptides.

Peptide	Sequence
Flu-TatC1	Flu-βAla-Tyr-Gly-Agp$_{49}$-Lys-Dab$_{51}$-Agp$_{52}$-Agp$_{53}$-Gln-Arg$_{55}$-Agb$_{56}$-Arg$_{57}$-NH_2
Flu-TatC2	Flu-βAla-Tyr-Gly-Agp$_{49}$-Lys-Dab$_{51}$-Agp$_{52}$-Agp$_{53}$-Gln-Arg$_{55}$-Agb$_{56}$-**Agb**$_{57}$-NH_2
Flu-TatC3	Flu-βAla-Tyr-Gly-Agp$_{49}$-Lys-Dab$_{51}$-Agp$_{52}$-Agp$_{53}$-Gln-Arg$_{55}$-**Agp**$_{56}$-Arg$_{57}$-NH_2
Flu-TatC4	Flu-βAla-Tyr-Gly-Agp$_{49}$-Lys-Dab$_{51}$-Agp$_{52}$-Agp$_{53}$-Gln-**Agp**$_{55}$-Agb$_{56}$-Arg$_{57}$-NH_2
Flu-TatC5	Flu-βAla-Tyr-Gly-Agp$_{49}$-Lys-Dab$_{51}$-Agp$_{52}$-**Arg**$_{53}$-Gln-Arg$_{55}$-Agb$_{56}$-Arg$_{57}$-NH_2
Flu-TatC6	Flu-βAla-Tyr-Gly-Agp$_{49}$-Lys-Dab$_{51}$-Agp$_{52}$-**Agb**$_{53}$-Gln-Arg$_{55}$-Agb$_{56}$-Arg$_{57}$-NH_2
Flu-TatC7	Flu-βAla-Tyr-Gly-Agp$_{49}$-Lys-**Dap**$_{51}$-Agp$_{52}$-Agp$_{53}$-Gln-Arg$_{55}$-Agb$_{56}$-Arg$_{57}$-NH_2

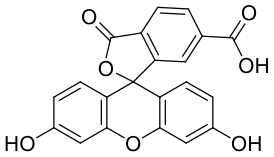
Flu = 6-carboxy-fluorescein; βAla = β-alanine



n=4 Agh, (*S*)-2-amino-6-guanidinohexanoic acid
n=3 Arg, L-arginine
n=2 Agb, (*S*)-2-amino-4-guanidinobutyric acid
n=1 Agp, (*S*)-2-amino-3-guanidinopropionic acid

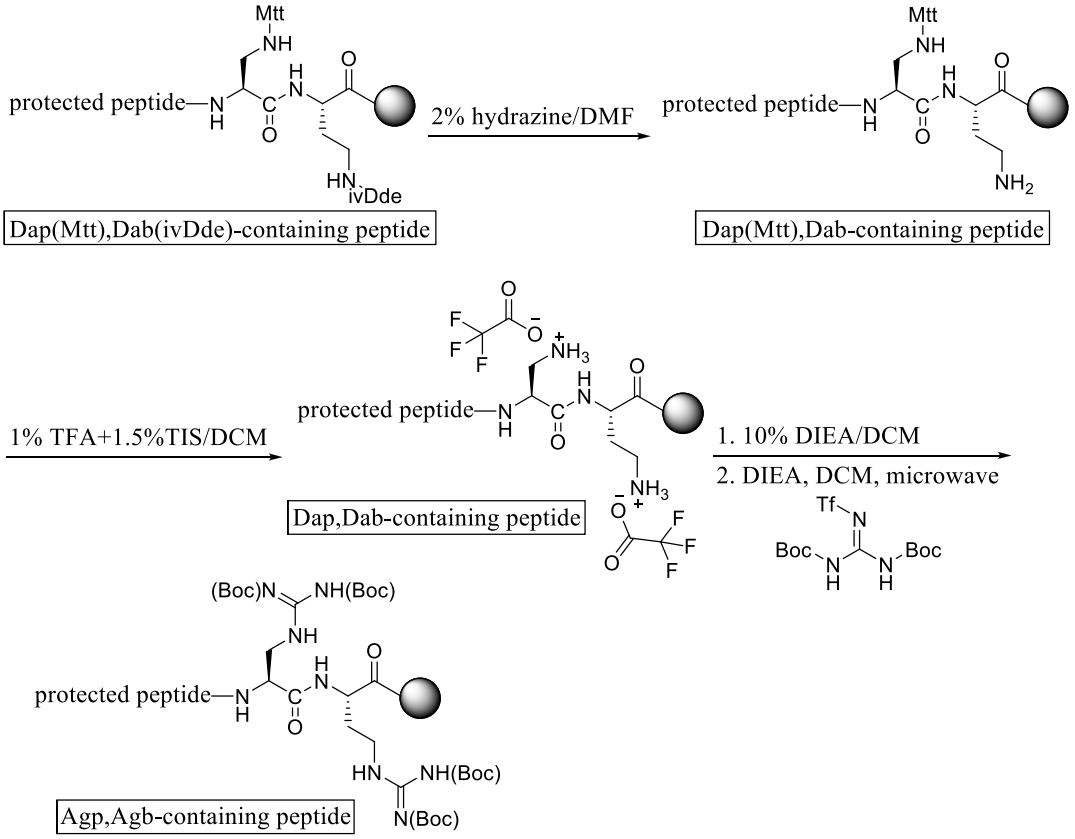


n=4 Lys
n=3 Orn
n=2 Dab
n=1 Dap



6-carboxy-fluorescein

Scheme S1. Synthesis of peptides containing both Agb and Agp.



Methods

General Materials and Methods

All of the chemical reagents except those indicated otherwise were purchased from Sigma-Aldrich. Diisopropylethylamine (DIEA), piperidine, trifluoroacetic acid (TFA), acetic anhydride, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide, Tween-20, and acetic anhydride were from Acros. Guanidine hydrochloride was from Fluka. Dimethylformamide (DMF), ethyl acetate, dichloromethane (DCM) and hexane were from Mallinckrodt. Methanol and acetonitrile were from Merck. Ammonium persulfate and 1,4-Dioxane were from J.T.Baker. Glycerol, boric acid, bis-acrylamide, Tris-HCl, and tris (hydroxylmethyl)-aminomethane (Tris) were from Bioshop. Organic and high performance liquid chromatography (HPLC) solvents were from Merck Taiwan. *N*-9-Fluorenylmethoxycarbonyl (Fmoc)-amino acids, 1-hydroxybenzotrazole (HOBt), and *O*-1*H*-benzotriazol-1-yl-1,1,3,3-tetra-methyl uronium hexafluorophosphate (HBTU) were from Novabiochem, Fmoc-PAL-PEG-PS resin was from Applied Biosystems. Primary antibody against Lamin B receptor was from Santa Cruz Biotechnology. Alexa Fluor 647-conjugated secondary donkey anti-goat antibody was from Life technologies. Reagents and solvents were used without further purification. Analytical reverse phase (RP)-HPLC was performed on an Agilent 1200 series chromatography system using a Vydac C_{18} column (4.6 mm diameter, 250 mm length). Preparative RP-HPLC was performed on a Waters Breeze chromatography system using a Vydac C_4 and C_{18} columns (22 mm diameter, 250 mm length). Mass spectrometry of the peptides was performed on a matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) spectrometer (Bruker Daltonics Biflex IV) using α-cyano-4-hydroxycinnamic acid as the matrix. Determination of peptide concentration was performed on a UV-Vis spectrophotometer (Jasco V-650). The gel shift results were imaged using a Typhoon TRIO$^+$ gel imager with the emission wavelength set at 526 nm. Cells were incubated using a CO_2 incubator (Thermo Scientific, Forma steri-cycle CO_2 incubaor). Cells were counted using a hemacytometer (Reichert Bright-Line, hemacytometer 1490). The fluorescence intensity of 6-carboxy-fluorescein labeled Tat peptides was measured on a flow cytometer (Becton Dickinson, FACS Canto^{TM} II) and the peptide-treated HeLa cells were imaged using a confocal microscope (TCS SP5, Leica).

_N_,_N_-Bis(tert-butoxycarbonyl)-guanidine

The synthesis was performed according to published procedures.[1-3] 1,4-Dioxane (30 mL) was added to a solution of guanidine hydrochloride (2.8727 g, 30.049 mmol) and sodium hydroxide (4.9573 g, 123.9 mmol) in water (30 mL), the mixture was cooled to 0 ^oC using an ice water bath. Di-*tert*-butyl-dicarbonate (14.5296 g, 66.1302 mmol) was then added to the reaction, and the residual was washed into the reaction with another 30 mL of 1,4-dioxane. The reaction was allowed to warm to room temperature and stirred for 3 days. The reaction mixture was then concentrated under reduced pressure to dryness. The resulting white emulsion was diluted with water (60 mL) and extracted with ethyl acetate (3 X 60 mL). The organic layer was then extracted with 10% citric acid (60 mL), water (60 mL), and brine (60 mL) and dried over anhydrous sodium sulfate. Finally, the dried organic solution was then concentrated under reduced pressure to obtain the desired product as a white powder (5.3942 g, 69.2% yield). ^1H NMR (400 MHz, $CDCl_3$) 3.46 (s, 1H), 1.46 (s, 18H); ESI-MS calculated for $C_{11}H_{21}N_3O_4$ [MH+]=260.3, observed [MH+]=260.1.

***N*,*N*'-Di-Boc-*N*"-trifluoromethanesulfonyl-guanidine**

The synthesis was performed according to published procedures.[1-3] A solution of *N*,*N*-bis(*tert*-butoxycarbonyl)-guanidine (3.0414 g, 12.035 mmole) and triethylamine (2.0 mL) in anhydrous dichloromethane (60 mL) was cooled to -68 ^{o}C using a dry ice/acetone bath under an atmosphere of nitrogen. Triflic anhydride (2.1 mL, 12.590 mmole) was added dropwise (2.1 mL/30 minutes). After half of the triflic anhydride was added, the color became light brown. The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction was washed with 2 M sodium bisulfate (20 mL) and water (20 mL), and then dried over anhydrous sodium sulfate. The dry organic solution was concentrated under reduced pressure and purified by chromatography on silica gel eluted with CH_2Cl_2 to obtained the desired product (3.0512 g, 64.8%). ^{1}H NMR (400 MHz, $CDCl_3$) 1.51 (s, 18H); ESI-MS calculated for $C_{12}H_{20}F_3N_3O_6S$ [MNa+]=414.4, observed [MNa+]=414.2.

N^{α}-Fmoc-(*S*)-2-amino-$N^{\omega,\omega'}$-di(Boc)-3-guanidinohexanoic acid (Fmoc-Agh(Boc)$_2$-OH).

The synthesis was performed following published procedures.[1] Fmoc-Lys-OH (1.100 g, 2.98 mmol) was suspended in anhydrous dichloromethane (6 mL) under nitrogen. *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (1.2 mL, 6.60 mmol) was added, and then the reaction mixture was heated to reflux until a clear solution was formed. The solution was cooled to room temperature, and *N,N'*-di-Boc-*N''*-trifluoromethanesulfonylguanidine [2, 3] (1.410 g, 3.60 mmol) was added followed by triethylamine (504 μL, 3.60 mmol). The reaction mixture was stirred at room temperature, and the reaction was monitored by TLC. Upon completion, the reaction mixture was diluted with dichloromethane (6 mL) and washed with 2 M sodium bisulfate and water, and dried with sodium sulfate. The dried organic solution was then concentrated under reduced pressure and purified by flash chromatography on silica gel (CH_2Cl_2 to 95:5 CH_2Cl_2/methanol) to obtain the desired product as a white powder (1.347g, 73.8% yield). R_f = 0.17 (95:5 CH_2Cl_2/methanol); m.p. 85-88° C; $[\alpha]^{25}_D$ = 16.9 (0.0099 g mL^{-1} $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$/TMS): δ = 8.439 (s, 1H), 7.749 – 7.257 (m, 8H), 5.638 (d, *J* (H,H) = 7.324 Hz, 1H), 4.505 (br s, 1H), 4.371 (d, *J* (H,H) = 5.798 Hz, 1H), 4.204 (t, *J* (H,H) = 6.867 Hz, 1H), 3.406 (m, 1H), 3.310 (m, 1H), 1.918 (m, 1H), 1779 (m, 1H), 1.479 (s, 9H), 1.471 (s, 9H), 1.647 – 1.277 ppm (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$/TMS): δ = 175.416, 163.388, 156.764, 156.604, 153.759, 144.425, 144.289, 141.830, 128.217, 127.602, 125.659, 120.477, 83.924, 80.395, 67.609, 54.064, 47.713, 41.187, 32.172, 29.008, 28.719, 28.567, 22.755 ppm; IR (liquid): ν bar = 3226, 2983, 1720, 1617, 1512, 1450, 1416, 1368, 1335, 1137, 1054 cm^{-1}; ESI-MS calculated for $C_{32}H_{42}N_4O_8$ [MH$^+$]: 611.3075, observed: 611.1; HRMS calculated for $C_{32}H_{42}N_4O_8$ [MH$^+$]: 611.3075, observed: 611.3063

Peptide Synthesis

Fmoc-PAL-PEG-PS (0.05 mmol) was swollen in *N, N*-dimethylformamide (DMF, 5 mL) for 30 minutes. The resin was then deprotected by 20% piperidine/DMF (3x8 min) and rinsed with DMF (5x1 min). A mixture of 3 equivalents of the appropriately protected Fmoc amino acid, HOBt and HBTU was dissolved in DMF (1 mL). Diisopropylethylamine (DIEA, 8 equivalents) was then added to the solution and mixed thoroughly. The solution was then applied to the resin. The vial that contained the solution was rinsed with DMF (1 mL) and added to the reaction. The first coupling was carried out for 8 hours. The 8th to the final residues were coupled for 1.5

hours. Other residues were coupled for 45 minutes. Arginine was triple coupled for 25 minutes each coupling. After each coupling, the resin was washed with DMF (5x1 min). For the acetyl capped peptides, a mixture of acetic anhydride (20 equivalents) and DIEA (20 equivalents) in DMF (3 mL) was applied to the resin and reacted for 2 hours. For peptides containg 6-carboxyfluorescein, a mixture of 6-carboxyfluorescein (3 equivalents), HOBt (3 equivalents), and HBTU (3 equivalents) was dissolved in 1 mL DMF. DIEA (8 equivalents) was then added to the solution, mixed thoroughly, and then applied to the resin. The residual solution in the vial was washed with DMF (2x1 mL), and applied to the resin and shaken for 6 hours. The resin was subsequently washed with DMF (5 mL, 5x1 min) and methanol (5 mL), and was lyophilized.

Solid phase guanidinylation was performed to synthesize the Agb-containing peptides.[1] For Agb-containing peptides, the corresponding Dab(ivDde)-containing peptide was synthesized first. The resin was treated with trityl chloride (12 equivalents, 2 x 16 hours) in 1 mL DCM to protect the fluorescein moiety.[4] Then the ivDde protecting group was removed by suspending the resin in 2% hydrazine in DMF (4 mL, 5 x 8 min) with shaking at room temperature. The resin was washed with DMF (4 mL, 5 x 1.5 min) and lyophilized. After removal of orthogonal protecting group from the resin-bound protected peptides, the resin was resuspended in a solution of *N,N'*-di-Boc-*N''*-trifluoro methanesulfonylguanidine (240 mg, 0.58 mmol) and DIEA (240 μL) in 1 mL CH_2Cl_2. The reaction was shaken under microwaved condition (20 sec/t, 70 W). The reaction was monitored until completion by cleaving a small amount (~5 mg) of peptide-bound resin and analyzed by RP-HPLC.

Solid phase guanidinylation was performed to synthesize the Agp-containing peptides.[5] For Agp-containing peptides, the corresponding Dap(Mtt)-containing peptide was synthesized first. Then the Mtt protecting group was removed by suspending the resin in 1% TFA and 1.5% TIS in DCM (2 mL, 20 x 1.5 min) with shaking at room temperature. The resin was washed with 10% DIEA in DCM (2 mL, 5 x 1 min) and lyophilized. The resin was resuspended in a solution of *N,N'*-di-Boc-*N''*-trifluoro methanesulfonylguanidine (240 mg, 0.58 mmol) and DIEA (240 μL) in 1 mL CH_2Cl_2. The reaction was shaken under microwaved condition (20 sec/t, 70 W). Reaction was monitored until completion by cleaving a small amount (~5 mg) of peptide-bound resin and analyzed by RP-HPLC.

Solid phase guanidinylation was performed to synthesize the peptides containing both Agb and Agp. For peptides containing both Agb and Agp, the corresponding peptide containing Dab and Dap with the orthogonal side chain protecting group 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde) on Dab and 4-methyltrityl (Mtt) on Dap was synthesized by SPPS, followed by selective removal of ivDde and Mtt and then solid phase guanidinylaion. The ivDde protecting group was selectively removed by 2% hydrazine in DMF first, following by Mtt removal using 1% trifluoroacetic acid (TFA) and 1.5% triisopropylsilane (TIS) in dichloromethane. Importantly, the resin was basified with 10% DIEA in DCM before solid phase guanidinylation. The resin was resuspended in a solution of *N,N'*-di-Boc-*N''*-trifluoro methanesulfonylguanidine (240 mg, 0.58 mmol) and DIEA (240 μL) in 1 mL CH_2Cl_2. The reaction was shaken under microwaved condition (20 sec/t, 70 W). The reaction was monitored until completion by cleaving a small amount (~5 mg) of peptide-bound resin and analyzed by RP-HPLC.

Peptides were deprotected and cleaved off the resin by treating the resin with 95:5 trifluoroacetic acid (950 μL)/triisopropylsilane (50 μL) with shaking for 2 hours. The solution was then filtered through glass wool and the resin was washed with TFA (3x1 mL). The combined filtrate was evaporated by a gentle stream of N_2. The resulting material was washed

with hexanes, dissolved in water, and lyophilized. The peptide (1 mg mL^{-1} aqueous solution) was analyzed using analytical RP-HPLC on a C18 column with a flow rate of 1 mL min^{-1}, temperature 25℃, linear 1 % min^{-1} gradient from 100% A to 0% A (solvent A: 99.9% water,0.1% TFA; solvent B: 90% acetonitrile, 10% water, 0.1% TFA). Appropriate linear solvent A/solvent B gradients were used for purification on preparative RP-HPLC on C_4 and C_{18} column. The identity of the peptide was confirmed by MALDI-TOF.

TatAgh57

(Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Agh-NH_2).

The peptide was synthesized using 266.3 mg (0.05059 mmol) of Fmoc-PAL-PEG-PS resin. The synthesis gave 379.3 mg of resin (75.3 % yield). The cleavage yielded 112.5 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 98.3% purity, using linear gradients PLG00_04 and PLG04_14, respectively. Retention time on analytical RP-HPLC was 17.1 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{67}H_{124}N_{33}O_{14}$ $[MH]^+$: 1615.00; observed m/z: 1615.36.

TatAgb57

(Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Agb-NH_2).

The corresponding TatDab57 peptide (Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Dab-NH_2) was synthesized using 266.0 mg (0.05053 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of TatDab57(ivDde) was 27.0 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{77}H_{136}N_{31}O_{16}$ $[MH]^+$: 1751.08; observed m/z: 1751.17. The ivDde protecting group was then selectively removed followed by solid-phase guanidinylation. The synthesis gave 419.8 mg of resin (99.8 % yield). The cleavage yielded 175.9 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 96.7% purity, using linear gradients PLG00_04 and PLG04_14, respectively. Retention time on analytical RP-HPLC was 16.9 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{65}H_{120}N_{33}O_{14}$ $[MH]^+$: 1586.97; observed m/z: 1586.93.

TatAgp57

(Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Agp-NH_2).

The corresponding TatDap57 peptide (Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Dap-NH_2) was synthesized using 266.8 mg (0.05068 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of TatDap57 was 16.5 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{63}H_{116}N_{31}O_{14}$ $[MH]^+$: 1531.79; observed m/z: 1531.01. The Mtt protecting group was then selectively removed followed by solid-phase guanidinylation. The synthesis gave 386.1 mg of resin (71.8 % yield). The cleavage yielded 83.4 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 96.8% purity, using linear gradients PLG00_04 and PLG05_14, respectively. Retention time on analytical RP-HPLC was 16.8 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{64}H_{118}N_{33}O_{14}$ $[MH]^+$: 1572.95; observed m/z: 1572.82.

TatAgh56
(Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Agh-Arg-NH_2).
The peptide was synthesized using 265.1 mg (0.05036 mmol) of Fmoc-PAL-PEG-PS resin. The synthesis gave 378.0 mg of resin (76.9 % yield). The cleavage yielded 166.2 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 98.8% purity, using linear gradients PLG00_04 and PLG04_14, respectively. Retention time on analytical RP-HPLC was 16.8 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{67}H_{124}N_{33}O_{14}$ $[MH]^+$: 1615.00; observed m/z: 1615.6.

TatAgb56
(Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Agb-Arg-NH_2).
The corresponding TatDab56 peptide (Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Dab-Arg-NH_2) was synthesized using 266.1 mg (0.05055 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of TatDab56(ivDde) was 26.0 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{77}H_{136}N_{31}O_{16}$ $[MH]^+$: 1751.08; observed m/z: 1751.53. The ivDde protecting group was removed and then solid-phase guanidinylation. The synthesis gave 413.0 mg of resin (88.8 % yield). The cleavage yielded 158.8 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 96.5% purity, using linear gradients PLG00_04 and PLG04_14, respectively. Retention time on analytical RP-HPLC was 16.9 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{65}H_{120}N_{33}O_{14}$ $[MH]^+$: 1586.97; observed m/z: 1587.36.

TatAgp56
(Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Agp-Arg-NH_2).
The corresponding TatDap56 peptide (Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Dap-Arg-NH_2) was synthesized using 267.9 mg (0.05089 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of TatDap56 was 16.7 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{63}H_{116}N_{31}O_{14}$ $[MH]^+$: 1531.79; observed m/z: 1531.99. The Mtt protecting group was then selectively removed followed by solid-phase guanidinylation. The synthesis gave 383.0 mg of resin (75.2 % yield). The cleavage yielded 124.7 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 96.4% purity, using linear gradients PLG00_04 and PLG05_15, respectively. Retention time on analytical RP-HPLC was 16.9 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{64}H_{118}N_{33}O_{14}$ $[MH]^+$: 1572.95; observed m/z: 1573.07.

TatAgh55
(Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Agh-Arg-Arg-NH_2).
The peptide was synthesized using 266.3 mg (0.05059 mmol) of Fmoc-PAL-PEG-PS resin. The synthesis gave 394.5 mg of resin (70.7 % yield). The cleavage yielded 145.2 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 98.5% purity, using linear gradients PLG00_04 and PLG05_14, respectively. Retention time on analytical RP-HPLC was 17.4 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{67}H_{124}N_{33}O_{14}$ $[MH]^+$: 1615.00; observed m/z: 1614.7.

TatAgb55
(Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Agb-Arg-Arg-NH_2).
The corresponding TatDab55 peptide (Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Dab-Arg-Arg-NH_2) was synthesized using 268.7 mg (0.05104 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of TatDab55(ivDde) was 24.8 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{77}H_{136}N_{31}O_{16}$ $[MH]^+$: 1751.08; observed m/z: 1750.77. The ivDde protecting group was removed and then solid-phase guanidinylation. The synthesis gave 377.4 mg of resin (65.1 % yield). The cleavage yielded 120.4 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 96.5% purity, using linear gradients PLG00_04 and PLG05_14, respectively. Retention time on analytical RP-HPLC was 17.1 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{65}H_{120}N_{33}O_{14}$ $[MH]^+$: 1586.97; observed m/z: 1587.51.

TatAgp55
(Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Agp-Arg-Arg-NH_2).
The corresponding TatDap55 peptide (Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Dap-Arg-Arg-NH_2) was synthesized using 264.2 mg (0.05019 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of TatDap55 was 16.0 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{63}H_{116}N_{31}O_{14}$ $[MH]^+$: 1531.79; observed m/z: 1530.78. The Mtt protecting group was then selectively removed followed by solid-phase guanidinylation. The synthesis gave 394.5 mg of resin (78.5 % yield). The cleavage yielded 128.9 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 96.4 % purity, using linear gradients PLG00_04 and PLG05_14, respectively. Retention time on analytical RP-HPLC was 16.6 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{64}H_{118}N_{33}O_{14}$ $[MH]^+$: 1572.95; observed m/z: 1572.45.

TatAgh53
(Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Agh-Gln-Arg-Arg-Arg-NH_2).
The peptide was synthesized using 223.0 mg (0.05129 mmol) of Fmoc-PAL-PEG-PS resin. The synthesis gave 337.9 mg of resin (67.1 % yield). The cleavage yielded 133.8 mg of crude peptide. The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 97.7% purity, using linear gradients PLG00_04 and PLG05_14, respectively. Retention time on analytical RP-HPLC was 17.1 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{67}H_{124}N_{33}O_{14}$ $[MH]^+$: 1615.00; observed m/z: 1614.9.

TatAgb53
(Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Agb-Gln-Arg-Arg-Arg-NH_2).
The corresponding TatDab53 peptide (Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Dab-Gln-Arg-Arg-Arg-NH_2) was synthesized using 220.4 mg (0.05069 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of TatDab53(ivDde) was 25.0 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{77}H_{136}N_{31}O_{16}$ $[MH]^+$: 1751.08; observed m/z: 1750.67. The ivDde protecting group was removed and then solid-phase guanidinylation. The synthesis gave 325.1 mg of resin (63.2 % yield). The cleavage yielded 114.5 mg of crude peptide. The peptide was purified by preparative RP-HPLC using C4 and C18

columns to 96.1% purity, using linear gradients PLG00_04 and PLG05_14, respectively. Retention time on analytical RP-HPLC was 16.8 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{65}H_{120}N_{33}O_{14}$ $[MH]^+$: 1586.97; observed m/z: 1586.45.

TatAgp53

(Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Agp-Gln-Arg-Arg-Arg-NH_2).

The corresponding TatDap53 peptide (Ac-Tyr-Gly-Arg-Lys-Lys-Arg-Dap-Gln-Arg-Arg-Arg-NH_2) was synthesized using 223.2 mg (0.05133 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of TatDap53 was 16.6 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{63}H_{116}N_{31}O_{14}$ $[MH]^+$: 1531.79; observed m/z: 1530.87. The Mtt protecting group was then selectively removed followed by solid-phase guanidinylation. The synthesis gave 334.7 mg of resin (65.7 % yield). The cleavage yielded 121.2 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 98.4 % purity, using linear gradients PLG00_04 and PLG06_14, respectively. Retention time on analytical RP-HPLC was 16.6 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{64}H_{118}N_{33}O_{14}$ $[MH]^+$: 1572.95; observed m/z: 1572.86.

TatAgh52

(Ac-Tyr-Gly-Arg-Lys-Lys-Agh-Arg-Gln-Arg-Arg-Arg-NH_2).

The peptide was synthesized using 221.7 mg (0.051 mmol) of Fmoc-PAL-PEG-PS resin. The synthesis gave 334.0 mg of resin (64.2 % yield). The cleavage yielded 133.3 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 97.7% purity, using linear gradients PLG00_04 and PLG05_14, respectively. Retention time on analytical RP-HPLC was 16.6 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{67}H_{124}N_{33}O_{14}$ $[MH]^+$: 1615.00; observed m/z: 1615.7.

TatAgb52

(Ac-Tyr-Gly-Arg-Lys-Lys-Agb-Arg-Gln-Arg-Arg-Arg-NH_2).

The corresponding TatDab52 peptide (Ac-Tyr-Gly-Arg-Lys-Lys-Dab-Arg-Gln-Arg-Arg-Arg-NH_2) was synthesized using 220.8 mg (0.05078 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of TatDab52(ivDde) was 25.0 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{77}H_{136}N_{31}O_{16}$ $[MH]^+$: 1751.08; observed m/z: 1751.72. The ivDde protecting group was removed and then solid-phase guanidinylation.The synthesis gave 324.1 mg of resin (62.2 % yield). The cleavage yielded 171.2 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 96.4% purity, using linear gradients PLG00_04 and PLG06_14, respectively. Retention time on analytical RP-HPLC was 16.3 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{65}H_{120}N_{33}O_{14}$ $[MH]^+$: 1586.97; observed m/z: 1587.47.

TatAgp52

(Ac-Tyr-Gly-Arg-Lys-Lys-Agp-Arg-Gln-Arg-Arg-Arg-NH_2).

The corresponding TatDap52 peptide (Ac-Tyr-Gly-Arg-Lys-Lys-Dap-Arg-Gln-Arg-Arg-Arg-NH_2) was synthesized using 219.0 mg (0.05037 mmol) of Fmoc-PAL-PEG-PS resin. Retention

time on analytical RP-HPLC of TatDap52 was 16.4 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{63}H_{116}N_{31}O_{14}$ $[MH]^+$: 1531.79; observed m/z: 1531.42. The Mtt protecting group was then selectively removed followed by solid-phase guanidinylation. The synthesis gave 334.1 mg of resin (69.1 % yield). The cleavage yielded 137.2 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 96.5% purity, using linear gradients PLG00_04 and PLG06_14, respectively. Retention time on analytical RP-HPLC was 16.7 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{64}H_{118}N_{33}O_{14}$ $[MH]^+$: 1572.95; observed m/z: 1572.10.

TatOrn51

(Ac-Tyr-Gly-Arg-Lys-Orn-Arg-Arg-Gln-Arg-Arg-Arg-NH_2).

The peptide was synthesized using 250.4 mg (0.050 mmol) of Fmoc-PAL-PEG-PS resin. The synthesis gave 381.1 mg of resin (77.7% yield). The cleavage yielded 93.8 mg of crude peptide (>99% yield). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 96.1% purity, using linear gradients PLG00_04 and PLG06_15, respectively. Retention time on analytical RP-HPLC was 16.8 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{65}H_{120}N_{33}O_{14}$ $[MH]^+$: 1586.97; observed m/z: 1586.47.

TatDab51

(Ac-Tyr-Gly-Arg-Lys-Dab-Arg-Arg-Gln-Arg-Arg-Arg-NH_2).

The peptide was synthesized using 249.2 mg (0.050 mmol) of Fmoc-PAL-PEG-PS resin. The synthesis gave 365.8 mg of resin (69.9% yield). The cleavage yielded 120.3 mg of crude peptide (>99% yield). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 96.0% purity, using linear gradients PLG00_04 and PLG06_15, respectively. Retention time on analytical RP-HPLC was 16.7 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{64}H_{118}N_{33}O_{14}$ $[MH]^+$: 1572.95; observed m/z: 1572.74.

TatDap51

(Ac-Tyr-Gly-Arg-Lys-Dap-Arg-Arg-Gln-Arg-Arg-Arg-NH_2).

The peptide was synthesized using 250.2 mg (0.050 mmol) of Fmoc-PAL-PEG-PS resin. The synthesis gave 372.0 mg of resin (73.1% yield). The cleavage yielded 123.0 mg of crude peptide (>99% yield). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 96.6% purity, using linear gradients PLG00_04 and PLG06_15, respectively. Retention time on analytical RP-HPLC was 16.8 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{63}H_{116}N_{33}O_{14}$ $[MH]^+$: 1558.94; observed m/z: 1559.28.

TatOrn50

(Ac-Tyr-Gly-Arg-Orn-Lys-Arg-Arg-Gln-Arg-Arg-Arg-NH_2).

The peptide was synthesized using 249.5 mg (0.050 mmol) of Fmoc-PAL-PEG-PS resin. The synthesis gave 383.3 mg of resin (79.8% yield). The cleavage yielded 99.8 mg of crude peptide (>99% yield). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 97.8% purity, using linear gradients PLG00_04 and PLG06_15, respectively. Retention time on analytical RP-HPLC was 16.8 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{65}H_{120}N_{33}O_{14}$ $[MH]^+$: 1586.97; observed m/z: 1586.58.

TatDab50

(Ac-Tyr-Gly-Arg-Dab-Lys-Arg-Arg-Gln-Arg-Arg-Arg-NH_2).

The peptide was synthesized using 249.6 mg (0.050 mmol) of Fmoc-PAL-PEG-PS resin. The synthesis gave 358.0 mg of resin (64.9% yield). The cleavage yielded 99.7 mg of crude peptide (>99% yield). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 98.1% purity, using linear gradients PLG00_04 and PLG06_15, respectively. Retention time on analytical RP-HPLC was 16.8 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{64}H_{118}N_{33}O_{14}$ $[MH]^+$: 1572.95; observed m/z: 1572.64.

TatDap50

(Ac-Tyr-Gly-Arg-Dap-Lys-Arg-Arg-Gln-Arg-Arg-Arg-NH_2).

The peptide was synthesized using 250.1 mg (0.050 mmol) of Fmoc-PAL-PEG-PS resin. The synthesis gave 383.9 mg of resin (80.3% yield). The cleavage yielded 106.2 mg of crude peptide (>99% yield). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 98.1% purity, using linear gradients PLG00_04 and PLG06_15, respectively. Retention time on analytical RP-HPLC was 16.8 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{63}H_{116}N_{33}O_{14}$ $[MH]^+$: 1558.94; observed m/z: 1558.43.

TatAgh49

(Ac-Tyr-Gly-Agh-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-NH_2).

The peptide was synthesized using 250.2 mg (0.05004 mmol) of Fmoc-PAL-PEG-PS resin. The synthesis gave 379.9 mg of resin (77.7 % yield). The cleavage yielded 141.7 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 98.4% purity, using linear gradients PLG00_04 and PLG05_14, respectively. Retention time on analytical RP-HPLC was 17.2 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{67}H_{124}N_{33}O_{14}$ $[MH]^+$: 1615.00; observed m/z: 1615.06.

TatAgb49

(Ac-Tyr-Gly-Agb-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-NH_2).

The corresponding TatDab49 peptide (Ac-Tyr-Gly-Dab-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-NH_2) was synthesized using 255.0 mg (0.05100 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of TatDab49(ivDde) was 28.8 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{77}H_{136}N_{31}O_{16}$ $[MH]^+$: 1751.08; observed m/z: 1751.99. The ivDde protecting group was removed and then solid-phase guanidinylation.The synthesis gave 379.5 mg of resin (74.6 % yield). The cleavage yielded 196.6 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 97.5 % purity, using linear gradients PLG00_04 and PLG06_14, respectively. Retention time on analytical RP-HPLC was 16.7 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{65}H_{120}N_{33}O_{14}$ $[MH]^+$: 1586.97; observed m/z: 1585.90.

TatAgp49

(Ac-Tyr-Gly-Agp-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-NH_2).

The corresponding TatDap49 peptide (Ac-Tyr-Gly-Dap-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-NH_2) was synthesized using 254.7 mg (0.0509 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of TatDap49 was 15.8 minutes. The identity of the peptide was

confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{63}H_{116}N_{31}O_{14}$ $[MH]^+$: 1531.79; observed m/z: 1530.85. The Mtt protecting group was then selectively removed followed by solid-phase guanidinylation.The synthesis gave 417.0 mg of resin (96.3 % yield). The cleavage yielded 135.3 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 97.1 % purity, using linear gradients PLG00_04 and PLG06_15, respectively. Retention time on analytical RP-HPLC was 16.4 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{64}H_{118}N_{33}O_{14}$ $[MH]^+$: 1572.95; observed m/z: 1573.01.

TatC1

(Ac-Tyr-Gly-Agp-Lys-Dab-Agp-Agp-Gln-Arg-Agb-Arg-NH_2).

The corresponding crude-TatC1 peptide (Ac-Tyr-Gly-Dap-Lys-Dab-Dap-Dap-Gln-Arg-Dab-Arg-NH_2) was synthesized using 224.3 mg (0.0404 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of crude-TatC1(ivDde) was 26.0 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{66}H_{114}N_{25}O_{16}$ $[MH]^+$: 1512.89; observed m/z: 1512.24. The ivDde and Mtt protecting group were removed and then solid-phase guanidinylation. The synthesis gave 313.5 mg of resin (72.5 % yield). The cleavage yielded 77.6 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 99.0 % purity, using linear gradients PLG00_01 and PLG05_14, respectively. Retention time on analytical RP-HPLC was 16.4 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{57}H_{104}N_{33}O_{14}$ $[MH]^+$: 1474.84; observed m/z: 1474.38.

TatC2

(Ac-Tyr-Gly-Agp-Lys-Dab-Agp-Agp-Gln-Arg-Agb-Agb-NH_2).

The corresponding crude-TatC2 peptide (Ac-Tyr-Gly-Dap-Lys-Dab-Dap-Dap-Gln-Arg-Dab-Dab-NH_2) was synthesized using 214.6 mg (0.0386 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of crude-TatC2(ivDde) was 37.0 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{77}H_{128}N_{23}O_{18}$ $[MH]^+$: 1662.98; observed m/z: 1662.71. The ivDde and Mtt protecting group were removed and then solid-phase guanidinylation. The synthesis gave 296.8 mg of resin (72.3 % yield). The cleavage yielded 73.1 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 98.6 % purity, using linear gradients PLG00_02 and PLG05_14, respectively. Retention time on analytical RP-HPLC was 16.4 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{56}H_{102}N_{33}O_{14}$ $[MH]^+$: 1460.83; observed m/z: 1461.05.

TatC3

(Ac-Tyr-Gly-Agp-Lys-Dab-Agp-Agp-Gln-Arg-Agp-Arg-NH_2).

The corresponding crude-TatC3 peptide (Ac-Tyr-Gly-Dap-Lys-Dab-Dap-Dap-Gln-Arg-Dap-Arg-NH_2) was synthesized using 222.0 mg (0.0400 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of crude-TatC3 was 14.7 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{52}H_{94}N_{25}O_{14}$ $[MH]^+$: 1293.46; observed m/z: 1292.57. The Mtt protecting group was removed and then solid-phase guanidinylation. The synthesis gave 308.3 mg of resin (70.1 % yield). The cleavage yielded 99.8 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and

C18 columns to 98.1 % purity, using linear gradients PLG00_02 and PLG05_14, respectively. Retention time on analytical RP-HPLC was 16.3 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{56}H_{102}N_{33}O_{14}$ $[MH]^+$: 1460.83; observed m/z: 1460.87.

TatC4

(Ac-Tyr-Gly-Agp-Lys-Dab-Agp-Agp-Gln-Agp-Agb-Arg-NH_2).

The corresponding crude-TatC4 peptide (Ac-Tyr-Gly-Dap-Lys-Dab-Dap-Dap-Gln-Dap-Dab-Arg-NH_2) was synthesized using 221.4 mg (0.0399 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of crude-TatC4(ivDde) was 25.6 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{63}H_{108}N_{23}O_{16}$ $[MH]^+$: 1442.83; observed m/z: 1442.49. The ivDde and Mtt protecting group were removed and then solid-phase guanidinylation. The synthesis gave 289.1 mg of resin (56.4 % yield). The cleavage yielded 95.0 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to >95 % purity, using linear gradients PLG04_05 and PLG05_14, respectively. Retention time on analytical RP-HPLC was 16.6 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{55}H_{100}N_{33}O_{14}$ $[MH]^+$: 1446.81; observed m/z: 1447.81.

TatC5

(Ac-Tyr-Gly-Agp-Lys-Dab-Agp-Arg-Gln-Arg-Agb-Arg-NH_2).

The corresponding crude-TatC5 peptide (Ac-Tyr-Gly-Dap-Lys-Dab-Dap-Arg-Gln-Arg-Dab-Arg-NH_2) was synthesized using 223.9 mg (0.0403 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of crude-TatC5(ivDde) was 26.1 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{69}H_{120}N_{27}O_{16}$ $[MH]^+$: 1582.94; observed m/z: 1582.50. The ivDde and Mtt protecting group were removed and then solid-phase guanidinylation. The synthesis gave 310.5 mg of resin (71.4 % yield). The cleavage yielded 109.9 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 99.1 % purity, using linear gradients PLG00_01 and PLG05_14, respectively. Retention time on analytical RP-HPLC was 16.1 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{59}H_{108}N_{33}O_{14}$ $[MH]^+$: 1502.87; observed m/z: 1502.18.

TatC6

(Ac-Tyr-Gly-Agp-Lys-Dab-Agp-Agb-Gln-Arg-Agb-Arg-NH_2).

The corresponding crude-TatC6 peptide (Ac-Tyr-Gly-Dap-Lys-Dab-Dap-Dab-Gln-Arg-Dab-Arg-NH_2) was synthesized using 223.4 mg (0.0402 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of crude-TatC6(ivDde) was 33.4 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{80}H_{134}N_{25}O_{18}$ $[MH]^+$: 1733.03; observed m/z: 1732.14. The ivDde and Mtt protecting group were removed and then solid-phase guanidinylation. The synthesis gave 308.5 mg of resin (70.3 % yield). The cleavage yielded 96.0 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 98.7 % purity, using linear gradients PLG00_02 and PLG05_14, respectively. Retention time on analytical RP-HPLC was 16.5 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{58}H_{106}N_{33}O_{14}$ $[MH]^+$: 1488.86; observed m/z: 1488.97.

TatC7

(Ac-Tyr-Gly-Agp-Lys-Dap-Agp-Agp-Gln-Arg-Agb-Arg-NH_2).

The corresponding crude-TatC7 peptide (Ac-Tyr-Gly-Dap-Lys-Dap-Dap-Dap-Gln-Arg-Dab-Arg-NH_2) was synthesized using 222.1 mg (0.0400 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of crude-TatC7(ivDde) was 25.7 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{65}H_{112}N_{25}O_{16}$ $[MH]^+$: 1498.87; observed m/z: 1498.68. The ivDde and Mtt protecting group were removed and then solid-phase guanidinylation. The synthesis gave 312.4 mg of resin (74.5 % yield). The cleavage yielded 92.5 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 98.5 % purity, using linear gradients PLG05_06 and PLG05_14, respectively. Retention time on analytical RP-HPLC was 16.4 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{56}H_{102}N_{33}O_{14}$ $[MH]^+$: 1460.83; observed m/z: 1461.57.

Flu-TatC1

(Flu-βAla-Tyr-Gly-Agp-Lys-Dab-Agp-Agp-Gln-Arg-Agb-Arg-NH_2).

The corresponding crude-Flu-TatC1 peptide (Flu-βAla-Tyr-Gly-Dap-Lys-Dab-Dap-Dap-Gln-Arg-Dab-Arg-NH_2) was synthesized using 223.8 mg (0.0403 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of crude-Flu-TatC1(ivDde) was 32.4 minutes. The ivDde and Mtt protecting group were removed and then solid-phase guanidinylation. The synthesis gave 283.8 mg of resin (47.9 % yield). The cleavage yielded 84.4 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 95.2 % purity, using linear gradients PLG02_11 and PLG17_26, respectively. Retention time on analytical RP-HPLC was 27.1 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{79}H_{117}N_{34}O_{20}$ $[MH]^+$: 1861.92; observed m/z: 1863.76.

Flu-TatC2

(Flu-βAla-Tyr-Gly-Agp-Lys-Dab-Agp-Agp-Gln-Arg-Agb-Agb-NH_2).

The corresponding crude-Flu-TatC2 peptide (Flu-βAla-Tyr-Gly-Dap-Lys-Dab-Dap-Dap-Gln-Arg-Dab-Dab-NH_2) was synthesized using 231.5 mg (0.0417 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of crude-Flu-TatC2(ivDde) was 41.2 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{99}H_{141}N_{24}O_{24}$ $[MH]^+$: 2050.05; observed m/z: 2051.16. The ivDde and Mtt protecting group were removed and then solid-phase guanidinylation. The synthesis gave 314.5 mg of resin (59.3 % yield). The cleavage yielded 106.6 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 95.6 % purity, using linear gradients PLG02_10 and PLG17_26, respectively. Retention time on analytical RP-HPLC was 27.2 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{78}H_{115}N_{34}O_{20}$ $[MH]^+$: 1847.90; observed m/z: 1848.7.

Flu-TatC3

(Flu-βAla-Tyr-Gly-Agp-Lys-Dab-Agp-Agp-Gln-Arg-Agp-Arg-NH_2).

The corresponding crude-Flu-TatC3 peptide (Flu-βAla-Tyr-Gly-Dap-Lys-Dab-Dap-Dap-Gln-Arg-Dap-Arg-NH_2) was synthesized using 223.7 mg (0.0403 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of crude-Flu-TatC3 was 26.8 minutes. The identity of the

peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{74}H_{107}N_{26}O_{20}$ $[MH]^+$: 1680.80; observed m/z: 1679.77. The Mtt protecting group was removed and then solid-phase guanidinylation. The synthesis gave 327.7 mg of resin (75.7 % yield). The cleavage yielded 121.0 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 96.7 % purity, using linear gradients PLG03_11 and PLG17_26, respectively. Retention time on analytical RP-HPLC was 27.3 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{78}H_{115}N_{34}O_{20}$ $[MH]^+$: 1847.90; observed m/z: 1850.25.

Flu-TatC4

(Flu-βAla-Tyr-Gly-Agp-Lys-Dab-Agp-Agp-Gln-Agp-Agb-Arg-NH_2).

The corresponding crude-Flu-TatC4 peptide (Flu-βAla-Tyr-Gly-Dap-Lys-Dab-Dap-Dap-Gln-Dap-Dab-Arg-NH_2) was synthesized using 224.0 mg (0.0403 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of crude-Flu-TatC4(ivDde) was 32.6 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{85}H_{120}N_{24}O_{22}$ $[MH]^+$: 1828.90; observed m/z: 1830.01. The ivDde and Mtt protecting group were removed and then solid-phase guanidinylation. The synthesis gave 334.8 mg of resin (84.8 % yield). The cleavage yielded 109.6 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 95.5 % purity, using linear gradients PLG02_10 and PLG17_26, respectively. Retention time on analytical RP-HPLC was 27.2 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{79}H_{117}N_{34}O_{20}$ $[MH]^+$: 1832.88; observed m/z: 1834.1.

Flu-TatC5

(Flu-βAla-Tyr-Gly-Agp-Lys-Dab-Agp-Arg-Gln-Arg-Agb-Arg-NH_2).

The corresponding crude-Flu-TatC5 peptide (Flu-βAla-Tyr-Gly-Dap-Lys-Dab-Dap-Arg-Gln-Arg-Dab-Arg-NH_2) was synthesized using 223.6 mg (0.0403 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of crude-Flu-TatC5(ivDde) was 32.4 minutes. The ivDde and Mtt protecting group were removed and then solid-phase guanidinylation. The synthesis gave 284.3 mg of resin (43.0 % yield). The cleavage yielded 71.2 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 96.1 % purity, using linear gradients PLG02_11 and PLG17_26, respectively. Retention time on analytical RP-HPLC was 27.1 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{81}H_{121}N_{34}O_{20}$ $[MH]^+$: 1889.95; observed m/z: 1891.54.

Flu-TatC6

(Flu-βAla-Tyr-Gly-Agp-Lys-Dab-Agp-Agb-Gln-Arg-Agb-Arg-NH_2).

The corresponding crude-Flu-TatC6 peptide (Flu-βAla-Tyr-Gly-Dap-Lys-Dab-Dap-Dab-Gln-Arg-Dab-Arg-NH_2) was synthesized using 224.6 mg (0.0404 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of crude-Flu-TatC6(ivDde) was 37.7 minutes. The ivDde and Mtt protecting group were removed and then solid-phase guanidinylation. The synthesis gave 287.7 mg of resin (45.6 % yield). The cleavage yielded 108.9 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 95.0 % purity, using linear gradients PLG02_11 and PLG17_26, respectively. Retention time on analytical RP-HPLC was 27.2 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{80}H_{119}N_{34}O_{20}$ $[MH]^+$: 1875.93; observed m/z: 1877.62.

Flu-TatC7

(Flu-βAla-Tyr-Gly-Agp-Lys-Dap-Agp-Agp-Gln-Arg-Agb-Arg-NH_2).

The corresponding crude-Flu-TatC7 peptide (Flu-βAla-Tyr-Gly-Dap-Lys-Dap-Dap-Dap-Gln-Arg-Dab-Arg-NH_2) was synthesized using 222.9 mg (0.0401 mmol) of Fmoc-PAL-PEG-PS resin. Retention time on analytical RP-HPLC of crude-Flu-TatC7(ivDde) was 32.7 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{87}H_{125}N_{26}O_{22}$ $[MH]^+$: 1885.95; observed m/z: 1886.98. The ivDde and Mtt protecting group were removed and then solid-phase guanidinylation. The synthesis gave 309.6 mg of resin (63.3 % yield). The cleavage yielded 98.2 mg of crude peptide (>99%). The peptide was purified by preparative RP-HPLC using C4 and C18 columns to 98.5 % purity, using linear gradients PLG03_11 and PLG17_26, respectively. Retention time on analytical RP-HPLC was 27.4 minutes. The identity of the peptide was confirmed by MALDI-TOF mass spectrometry. Calculated for $C_{78}H_{115}N_{34}O_{20}$ $[MH]^+$: 1847.90; observed m/z: 1848.96.

UV-vis Spectroscopy for Peptide Concentration Determination

The concentration of the TatXaa*N*, TatZbb*N* and TatCX peptide stock solutions were determined using the Edelhoch method.[6, 7] An approximately 10 mM stock solution was prepared for each TatXaa*N*, TatZbb*N* and TatCX peptide based on weight. The UV data was collected using a 1 mm pathlength cell. The concentration of TatXaa*N*, TatZbb*N* and TatCX peptide stock solutions was determined by the tyrosine absorbance in 6 M guanidinium chloride (ε_{282} = 1220, ε_{280} = 1285, ε_{278} = 1395, ε_{276} = 1455).[6, 7] The UV absorbance was collected at wavelengths 276, 278, 280, and 282 nm over 1 minute (60 x 1 sec) to accurately determine the concentration of the sample. The concentration of the peptide solutions was derived using Kaleidagraph 3.52 (Synergy Software, CA).

An approximately 7 mM stock solution was prepared for each Flu-TatZbb51 and Flu-TatCX peptide based on weight. The UV data was collected using a 1 mm pathlength cell. The concentration of Flu-TatZbb51 and Flu-TatCX peptide stock solutions was determined in pH 9 buffer (1 mM sodium phosphate, 1 mM sodium citrate, and 1 mM sodium borate) based on the absorbance of 6-carboxyfluorescein (ε_{492} = 81000).[8] The UV absorbance was collected at 492 nm over 1 minute (60 x 1 sec) to accurately determine the concentration of the sample based on the Beer–Lambert law.[9] The concentration of the peptide stock solutions was derived using Kaleidagraph 3.52 (Synergy Software, CA).

Electrophoretic Mobility Shift Assay

The fluorescein-labeled TAR RNA was purchased from Sigma. The fluorescein-labeled RNA was dissolved in diethyl pyrocarbonate treated H_2O to give a 50 μM solution. Binding assays were performed at room temperature. Peptide and RNA were incubated in pH 7.4 buffer (10 μL) containing Tris-HCl (50 mM), KCl (50 mM), 2% glycerol, and Triton X-100 (0.05%) in the presence or absence of poly-dIdC (10 μg/mL) or bulk *E. coli* tRNA (10 μg/mL) for 30 minutes. The RNA concentration was 100 nM. The samples were analyzed by loading into 12%

native polyacrylamide gels in 0.5% TB buffer and electrophoresis was performed with 140 V at room temperature. Dried gels were scanned by the Typhoon TRIO+ Variable Mode Imager. Bands corresponding to the free (unbound) and bound RNA were quantified using the ImageQuant software. The fraction bound RNA was derived by dividing the band intensity of bound RNA by the sum of the band intensities for the free (unbound) and bound RNA. The apparent dissociation constants were globally derived from the quantified data assuming a 1:1 binding stoichiometry using the following equation in Kaleidagraph 3.52 (Synergy Software, CA).

$$Fraction\ Bound\ RNA = \frac{\left([RNA]_{Total} + [Peptide]_{Total} + K_D - \sqrt{\left([RNA]_{Total} + [Peptide]_{Total} + K_D \right)^2 - 4[RNA]_{Total} \cdot [Peptide]_{Total}} \right)}{2[RNA]_{Total}}$$

where $[RNA]_{Total}$ is the total RNA concentration (including unbound RNA and bound RNA), $[Peptide]_{Total}$ is the total peptide concentration (including unbound peptide and bound peptide, and K_D is the apparent dissociation constant for the RNA-peptide complex.

Cellular Uptake Assay

All apparatuses were sterilized by an autoclave sterilizer, and the experimental surfaces were wiped with 70% ethanol. All operations were performed in a laminar flow hood. The number of cells was determined by a hemacytometer. There were 9 squares with 1.0 mm^2 area and 0.1 mm depth in the hemacytometer. The number of cells in the four corner squares was counted and averaged. The average was multiplied by 10^4 to obtain the number of cells in a 1 mL suspension. The cellular uptake experiments were performed using 6 x 10^5 cells. Jurkat cells were incubated with the peptides at 7 and 120 μM at 37 ˚C with 5% CO_2 for 15 minutes at 37 ˚C. For ATP depleted conditions, cells incubated with ATP inhibitors sodium azide (10 mM) and 2-deoxy-D-glucose (6 mM) for 1 hour followed by incubation with Flu-TatCX at 7 μM in the presence of two ATP inhibitors for 15 minutes at 37˚C with 5% CO_2. The cells were then washed with PBS (2g/L KCl, 2g/L KH_2PO_4, 80g/L NaCl, 11.5g/L Na_2PO_4) (2 x 400 μL) to remove the fetal bovine serum which might interfere with the proteolytic activity of trypsin. The cells were then incubated with 0.05 % trypsin/EDTA in PBS for 5 minutes to remove the peptides which adhered to the cell surface rather than entered into the cell.[10] The cells were washed with PBS (2 x 400 μL). The extracellular fluorescence quencher, trypan blue, was added to the cell suspension to quench the fluorescence from the peptides adsorbed onto the outside of the cell.[11, 12] The cells were terminated by adding Triton-X 100 to give the dead control group. Propidium iodide (PI) was added to all samples to stain the dead cells but should not stain the live cells. Fluorescence analysis for the Jurkat cells was performed with a flow cytometer (FACScan, Becton Dickinson Bioscience). The voltage of the photomultiplier tube for forward scatter, side scatter, and propidium iodide was set to 235, 450, and 260, respectively. Live cells containing appropriate forward scatter and side scatter values were selected and gated as the P1 region for normal and live cells in the live control group. The minimum propidium iodide fluorescence intensity for the dead cells treated with propidium iodide in the P1 region was set as the threshold value for dead cells. In other words, cells with propidium iodide fluorescence below the threshold value would be deemed live cells. The fluorescence of 6-carboxyfluorescein was

considered when the cell morphology was in the P1 region and the propidium iodide fluorescence intensity was lower than the threshold value. The 6-carboxyfluorescein fluorescence intensity was acquired for 10,000 events at room temperature. The data presented are the mean fluorescence intensity for the 10,000 cells. Each experiment was independently repeated at least three times.

Fluorescence Confocal Microscopy Imaging

$1x10^5$ HeLa cells per well were seeded in 6-well plates and incubated in a DMEM medium supplemented with 10% serum for 24 hours. The HeLa cells were then incubated with 10 μM Flu-TatCX peptides for 4 hours at 37 ^oC in DMEM medium with fetal bovine serum, followed by washing with PBS three times and fixing for 10 minutes in 4 % paraformaldehyde in PBS. Cells were then permeabilized in 0.1% w/v Triton X-100 in PBS and stained with DAPI. The cells were washed with PBS and analyzed by confocal microscopy (TCS SP5, Leica).

Nuclear Localization Imaging by Fluorescence Confocal Microscopy

$1x10^5$ HeLa cells per well were seeded in 6-well plates and incubated in a DMEM medium supplemented with 10% serum for 24 hours. The HeLa cells were then incubated with 10 μM Flu-TatCX peptides for 24 hours at 37 ^oC in DMEM medium with fetal bovine serum, followed by washing with PBS three times and fixing for 10 minutes in 4 % paraformaldehyde in PBS. Cells were then permeabilized in 0.1% w/v Triton X-100 in PBS. The cells were incubated with the primary antibody against Lamin B receptor (1:400) overnight. After washing, the bound primary antibodies were labeled with Alexa Fluor 647-conjugated secondary anti-goat antibody (1:300). The cells were then washed with PBS and analyzed by confocal microscopy (TCS SP5, Leica).

Quantitative Nuclear Localization by Fluorescence Confocal Microscopy

$1x10^5$ HeLa cells per well were seeded in 6-well plates and incubated in a DMEM medium supplemented with 10% serum for 24 hours. The HeLa cells were then incubated with 10 μM Flu-TatC1 amd Flu-TatC4 peptides for 24 hours at 37 ^oC in DMEM medium with fetal bovine serum, followed by washing with PBS three times and fixing for 10 minutes in 4 % paraformaldehyde in PBS. Cells were then permeabilized in 0.1% w/v Triton X-100 in PBS. The cells were incubated with the primary antibody against Lamin B receptor (1:400) overnight. After washing, the bound primary antibodies were labeled with Alexa Fluor 647-conjugated secondary anti-goat antibody (1:300). The cells were washed with PBS and analyzed by confocal microscopy (TCS SP5, Leica). The green fluorescence (peptide) within the boundary of red fluorescence (lamin B) was calculated and integrated to quantify the amound of peptide in the nucleus using METAMORPH.[13] The quantitative data was acquired from more than 50 cells.

Proteolysis Resistance Assay

The peptides ArgTat and TatCX at 0.5 mM were treated with trypsin (0.1 mg/mL) in 10 mM Tris solution at pH 7.5. At different time points upon incubation with trypsin, 50 μL aliquots of the reaction mixture were dilated into 100 μL 5% TFA aqueous solution to terminate the reaction. These reactions were quantified by reverse phase analytical HPLC monitoring the absorbance at 220 nm and the peaks were identified by MALDI-TOF mass spectrometry. The half-life of the peptides was determined by plotting the percent remaining peptide versus time, and fitting the data using Kaleidagraph 3.52 (Synergy Software CA).

Intracellular Tat-Dependent Luciferase Expression Assay

1 x 10^5 2F5 cells were transferred into a 96-well plate and pre-incubated separately with ArgTat and TatCX peptides at 100 μM for 24 hours at 37 °C in RPMI medium with 10% FBS serum. After incubation, the activator (SAHA) was added (to 0.9 μM) in the presence of peptides for another 14 hours. The cells were then washed with PBS to remove the peptides. The cells were treated with lysis buffer and luciferase activity was determined by a luciferase assay (Promega E6110). The cells showed minimally detectable reporter gene expression prior to activation by SAHA. The cells that were not treated with SAHA served as the negative control and the cells that were treated with SAHA served as the positive control. The luminescence intensity for the data was derived by subtracting the luminescence of the negative control from the raw data. The luminescence was measured using a microplate hybrid reader (BioTek Synergy H1).

Cell Viability Assay

4x10^4 Jurkat cells per well were transferred into 96-well plates. The cells were incubated with 120 μM of ArgTat and TatCX for 24 hours, followed by incubation with the WST-1 reagent (1:10 final dilution) for another 30 minutes at 37°C. The absorbance at 450 nm of the solution was measured, with a reference wavelength of 650 nm, using a microplate reader (Bio-Rad, model 680). The yellow-colored formazan dye generated by the live cells was proportional to the number of live cells.

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